Pathovar identification and genetic diversity of *Xanthomonas translucens* strains isolated from weedy grasses and cultivated wild rice in Minnesota

A THESIS
SUBMITTED TO THE FACULTY OF THE
UNIVERSITY OF MINNESOTA
BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE

Ruth Dill-Macky and Carol Anne Ishimaru, Advisors

September 2019

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Acknowledgements

Thank you to those who have helped me, guided me, and contributed to my Master's graduate program. I grew as a student, as an aspiring plant pathologist, and as an individual because of the strong support group I had alongside me during this journey.

It is with all my heart that I share my gratefulness to my advisors Dr. Ruth Dill-Macky and Dr. Carol Ishimaru. I was very blessed to have the opportunity to be advised by not one but two inspiring women Plant Pathologists. I thank them for the never-ending encouragement, for "making me" participate in more public speaking events than I might have preferred, and for always providing me with confidence in my coursework, research, and everyday life. There were many great moments of joy and excitement, but also moments of worry and stress. My advisors have a magical way of turning my worries into inspiration with their understanding and compassion. I could spend the rest of the night highlighting the great attributes of Dr. Dill-Macky and Dr. Ishimaru and how they contributed to my development as a scientist, but they would likely advise me to get some sleep.

I would also like to express my appreciation to Dr. Jeff Gunsolus, who played an active role in serving on my committee. I thank him for participating in all the committee meetings and providing insightful feedback to my research. I am thankful for his time and willingness to answer questions when they arose.

A special thanks to Rebecca Curland for always being there to teach and support me. Rebecca has been an exceptional mentor, unofficial committee member, and collaborator. Her guidance, from day one, has allowed me to improve my research and communication skills from laboratory techniques to posters, presentations, and writing.

I especially thank Beheshteh Zargaran and Zennah Kosgey for technical and moral support. Also, the handfuls of Small Grains Pathology undergraduate students and interns that assisted in lab, field, and greenhouse experiments. Their time, support, and comradery were very valuable to this work.

Thanks to Dan Braaten for assisting in the collection of plant samples in Minnesota cultivated wild rice fields.

Dedication

This thesis is dedicated to my loving parents, David and Lisa Ledman. Thank you for always supporting me, but especially through this special time as I continue to follow my passion for plant pathology. Thank you for watching the dogs during late nights and when I was out of town for conferences or field work. I also dedicate this thesis to my brother, Justin Ledman, who provided support all the way from Alaska.

Abstract

Bacterial leaf streak (BLS) of wheat, caused by *Xanthomonas translucens* pv. *undulosa*, has been prevalent in Minnesota wheat fields for the past decade. Infested seed and crop debris can serve as inoculum for subsequent crops. Weeds are also considered a potential inoculum source, but little is known about specific pathovars and genetic diversity among strains of *X. translucens* from these different hosts. The objectives of this study were to isolate *X. translucens* from poaceous weeds and cultivated wild rice in Minnesota, determine pathogenicity of these strains on wheat and barley, assess phylogenetic relationships and genetic diversity of strains using multilocus sequence analysis (MLSA) and typing (MLST) of four housekeeping genes (*rpoD*, *dnaK*, *fyuA*, and *gyrB*), and evaluate the efficacy of loop-mediated isothermal amplification (LAMP) assays designed to identify *X. translucens* pathovars that cause BLS on small grains. Bacteria were isolated from 157 plant samples, representing 12 poaceous hosts collected in and around commercial fields of wheat and wild rice. Strains exhibiting characteristic colony morphology on Wilbrink's medium were purified and evaluated further. The majority (87/134) of *Xanthomonas* strains were predicted to be *X. translucens* by 16S rDNA sequencing. A subset (51) of the strains predicted to be *X. translucens* were infiltrated into leaves of wheat and barley seedlings and found to cause disease. Eight of these strains were also tested in the field and likewise caused disease on wheat and barley. Phylogenies from MLSA show that strains from weedy grasses and wild rice are closely related to known *X. translucens* pathovars, most commonly *X. translucens* pv. *undulosa*. The findings suggest that poaceous weeds and cultivated wild rice have potential to serve as reservoirs of inoculum for the bacterial pathogen inciting BLS of wheat.

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Chapter 1 . Literature Review of Bacterial Leaf Streak

1.1 Introduction

Bacterial leaf streak (BLS), caused by *Xanthomonas translucens* (Jones et al. 1917) Vauterin et al. 1995, is a widespread but sporadic disease affecting small grains. As the name indicates, the disease is caused by a bacterium and results in chlorotic and necrotic, translucent streaks on leaf blades. Recent BLS outbreaks in the Upper Great Plains, including Minnesota, North Dakota, and South Dakota, have been of major concern to wheat (*Triticum aestivum* L.), and barley (*Hordeum vulgare* L.), growers over the last decade (Adhikari et al. 2011; Curland et al. 2018). In 2017, the United States produced 1.7 billion bushels of wheat (including winter, spring, and durum wheat) and 140 million bushels of barley; Minnesota and the Dakotas, combined, accounted for 18% of total U.S. wheat production and 22% of U.S. barley production (USDA 2017). Minnesota produces almost exclusively hard red spring wheat (99.7% in 2017), which is 20% of the nation's total hard red spring wheat production (USDA 2017). Under favorable conditions, the disease progresses rapidly and causes economic loss. Few studies have calculated losses caused by BLS, but yield losses in wheat up to 40% were reported in Idaho (Forster 1982) and a 10.5% reduction of kernel weight was measured in susceptible wheat varieties (Waldron 1929).

Xanthomonas translucens has undergone multiple taxonomic changes during the exploration of its characteristics and host specializations. Four pathovars of *X. translucens* are currently recognized to cause disease on small grains and are differentiated at the infrasubspecific level based on variation in the pathogen's host range (Bull et al. 2010; Dye et al. 1980). The pathovar strains are indistinguishable using

classic physiological and cultural techniques. Until recently, a lack of methods to identify the pathogen using molecular and genetic tools resulted in a confusing taxonomic history of *Xanthomonas* species. Methods such as 16S rDNA sequencing, multilocus sequence analysis (MLSA), multilocus sequence typing (MLST), and loop-mediated isothermal amplification (LAMP) have aided in the identification and characterization of closely related *X. translucens* strains in recent years (Adhikari et al. 2012; Curland et al. 2018; Hauben et al. 1997; Langlois et al. 2017; Young et al. 2008).

1.2 Symptoms and Signs

BLS affects a number of hosts and although the symptoms and signs of BLS are similar on all hosts some variation in disease progression and severity occur (Wallin 1946). Differences in symptomology are influenced by the environment, host variety, pathogen strain, and interactions of the plant with other pathogens (Bamberg 1936; Boosalis 1952). Within the variation, characteristic symptoms and signs are recognized in BLS infection.

Bacteria enter leaf tissue through stomata, hydathodes, or wounds. Initial symptoms appear on young leaf tissue as small water-soaked lesions, dark green in color, and easily overlooked by the untrained eye (Bamberg 1936; Jones et al. 1917). Lesions typically begin mid-leaf, where the blade naturally bends. Moisture from morning dew or rainfall settles on this portion of the leaf and promotes infection (Duveiller 1994a). Water-soaked regions expand longitudinally, initially limited by leaf veins, creating visible streaks along the blade. Streaks progress from the point of infection toward the leaf tip and down to the leaf sheath. Infections expand laterally when the striped lesions

coalesce, bypassing the vein restriction. Lesions become chlorotic and develop a transparent, greasy appearance. This characteristic translucency remains evident as infection progresses. Expanded lesions become necrotic, turning from yellow to brownish-black (Bamberg 1936; Jones et al. 1917). Infections spreading across the entire width of the leaf blade will cause tissue distal to the lesion to wilt and die (Bamberg 1936).

The symptoms may progress to cover all other above ground plant parts. Symptoms on the stem are evident as water-soaked streaks or dark sunken lesions. Streaked lesions on the stems may be thin and dispersed, or symptoms may merge to wrap around the entire culm or rachis. Infected glumes show similar symptoms of water-soaking and dark, longitudinal streaking or sunken lesions (Bamberg 1936; Smith et al. 1919). The awns may appear greasy with alternating bands of dark discoloration (Duveiller 1994b). Due to the darkening of the glumes, infection of the heads is often referred to as black chaff disease. Severe infection may cause the heads to be stunted and distorted and may result in kernels being aborted. Kernels are not always entirely lost, so symptomatic kernels may be darkened, stunted, and shriveled in a honeycomb pattern (Jones et al. 1917; Smith et al. 1919).

Under moist conditions, bacterial ooze may exude from fresh lesions. Milky droplets dry around lesions as hard yellowish granules, or the ooze may spread over the leaf surface drying into a thin greyish, flaky film (Bamberg 1936; Smith et al. 1919). Severe flag leaf infections may produce enough bacterial exudate to seal the leaf sheath and prevent head emergence (Jones et al. 1917). Bacterial ooze may also be present on infected glumes and in pockets on shriveled kernels, but it is reported to be less evident

than signs on leaf tissue (Smith et al. 1919). Bacterial streaming, as observed under the microscope from sliced sections of infected tissue mounted with water is characteristic of BLS infections. As described by Smith et al. (1919), the bacterial plume from the diseased tissue into the water "like smoke out of a chimney."

1.3 Pathogen Characteristics

Members of the *Xanthomonas* genus are Gram-negative, non-sporing, and strictly aerobic. The bacterium is a cylindrical rod with rounded ends 0.5-0.8 x 1.0-2.5 µm in size and motile by a single polar flagellum (Dowson 1939; Jones et al. 1917). Colony and cell morphology along with physiological tests can be used to help identify the pathogen to genus and species levels, but these tests cannot be used to distinguish between pathovars (Duveiller et al. 1997; Dye 1962; Dye and Lelliott 1974).

In culture, the bacteria form round, convex, viscous, yellow colored colonies that produce extracellular polysaccharides (EPS) when glucose is available. The yellow pigments, known as xanthomonadins, are membrane bound, not water-soluble, and unique to *Xanthomonas* species (Schaad and Stall 1988; Starr et al. 1977). The EPS produced by *Xanthomonas* species is used to make xanthan gum, commonly used in food production (Jeanes et al. 1961).

Colony characteristics may vary slightly by strain, but all will produce distinct yellow, sticky, gelatinous colonies on most solid media (Dowson 1939; Jones et al. 1917). When isolating the pathogen from plant material, optimal temperature for rapid growth is 25-27°C, but the organism is reported to grow between a wide range of

temperatures, 7-40°C (Bamberg 1936; Dye and Lelliott 1974). Cultural characteristics along with physiological information can help distinguish xanthomonads from saprophytic or other bacteria present (Duveiller et al. 1997).

1.4 Overwintering and Dissemination

The source of primary inoculum often derives from the location where the pathogen overwinters. Pathogen survival during winter months is especially important in regions where weather conditions are particularly harsh, such as the northern climates of North America including the states of Minnesota, North Dakota, and South Dakota. The BLS organism is highly resistant to cold temperatures and cycles of freezing and thawing. Bamberg (1936) demonstrated the pathogen's ability to withstand a typical Midwest winter, surviving outdoor conditions for four months with temperatures fluctuating between –33.3°C and 20.0°C.

The pathogen can overwinter in seed, winter cereal crops, crop debris, and perennial weeds. Diseased seedlings have been observed in the subsequent crop when seeds of infected hosts were used for planting (Boosalis 1952; Braun 1920). The rate of transmission of bacteria overwintering in seed to seedling is variable. Braun (1920) observed 82% disease incidence while Boosalis (1952) observed only 3-6% incidence, though these reports suggest that seed transmission is still an effective mode of survival and dispersal. Long distance dissemination of the bacteria occurs when infected seed is distributed to new locations (Jones et al. 1917). The pathogen is highly resistant to desiccation when in association with plant material, which increases its chance of survival. Jones et al. (1917) isolated viable bacteria from seed stored for two years and

from leaf tissue that had dried for eight months, but bacteria allowed to dry in the absence of plant material died within one day. Similarly, Boosalis (1952) found the pathogen could not survive in soil that was free of plant debris, but he reported obtaining diseased seedlings when infected straw was placed on the surface of the soil. This suggests that plant material, even when dried, protects the bacteria from total desiccation.

The wide host range of some *X. translucens* pathovars includes grass species that are common weeds in and around small grain fields, such as smooth brome (*Bromus inermis* Leyss.) and quackgrass (*Elymus repens* (L.) Gould). During the springtime, Wallin (1946) observed infected smooth brome seedlings emerging from beneath the previous season's dead leaf matter. Bacteria were isolated from both the new and old tissue and was pathogenic on smooth brome, wheat, barley, and rye (*Secale cereale* L.). Boosalis (1952) supported Wallin's findings and attained the same results on infected quackgrass. Local dissemination of *X. translucens* occurs primarily by splashing water and plant-to-plant contact. Smooth brome and quackgrass are some of the first plants to emerge in the spring and bacteria from infested plants have the potential to infect nearby small grains (Boosalis 1952; Wallin 1946). Little to no exploration of annual grass species hosting *X. translucens* strains, either as pathogens or epiphytes, has occurred.

1.5 Taxonomic History

BLS was initially observed in the early 1900's on barley and wheat grown in the United States' Upper Great Plains. The causal agent, *X. translucens,* infects many species in the Poaceae family, including some of the small grains crops along with a range of grasses (Hagborg 1942; Jones et al. 1917; Reddy et al. 1924; Smith et al. 1919).

Over the last century, multiple taxonomic changes took place. *X. translucens* pv. *cerealis* (Hagborg 1942), *X. translucens* pv. *secalis* (Reddy et al. 1924), *X. translucens* pv. *translucens* (Jones et al. 1917), and *X. translucens* pv. *undulosa* (Smith et al. 1919) Vauterin et al. 1995 are the currently recognized pathovars that cause BLS on small grains (Bull et al. 2010).

First identified on barley, the bacterium was described by Jones et al. (1917) as *Bacterium translucens*, a name that signified the translucent symptoms observed on leaf blades. Host range studies on barley, wheat, three other *Triticum* spp., rye, oats, and timothy demonstrated that symptom development from *B. translucens* occurred only on barley (Jones et al., 1917).

Similar symptoms were reported on wheat and Smith et al. (1919) described the bacterium as morphologically and symptomatically indistinguishable from the organism found on barley except that it was pathogenic on wheat, barley, and rye, suggesting a significant biological difference. This organism was named *Bacterium translucens* var. *undulosum*, and thus was considered a variant of the barley pathogen (Smith et al., 1919). This strain was reported to have a wider host range than the strain found on barley. Bamberg (1936) expanded the host range study and in addition to wheat, barley, and rye found *B. translucens* var. *undulosum* caused typical symptoms on some oat (*Avena sativa* L.) varieties, smooth brome, and foxtail barley (*Hordeum jubatum* L.). In related studies, oats showed no infection when inoculated with *B. translucens* var. *undulosum* (Hagborg 1942; Jones et al. 1916). Inconsistencies in the results of studies testing oat susceptibility may have been attributed to the use of resistant oat varieties or pathogen strains not virulent on oats, when infection did not occur (Bamberg 1936).

Reddy et al. (1924) discovered a previously undescribed strain on rye, naming it *Bacterium translucens* var. *secalis*. This strain was reported to be pathogenic only on rye, which distinguished it from the other *B. translucens* pathogens previously described. This strain had identical symptomology, morphology, and growth in culture to both *B. translucens* and *B. translucens* var. *undulosum* and could only be differentiated by its host specialization (Reddy et al. 1924).

Undergoing multiple taxonomic revisions, the genus *Bacterium* was replaced with the currently accepted genus *Xanthomonas* (Dowson 1939). With acceptance of *Xanthomonas* as the genus name, Hagborg (1942) recommended the use of *forma specialis* (f. sp.) to describe strain differences versus the use of variant (var.) and suggested five *forma specialis* of the *X. translucens* pathogen based on their ability to infect barley, wheat, oats, and rye. Following this nomenclature, the organism found most commonly on barley, *X. translucens* f. sp. *hordei*, was reported to only be pathogenic on barley; *X. translucens* f. sp. *undulosa*, generally found most commonly on wheat and rye, was defined by being pathogenic on wheat, barley, and rye; *X. translucens* f. sp. *secalis*, found on rye, was reported to only be pathogenic on rye; *X. translucens* f. sp. *hordei-avenae*, most commonly found on barley, was reported to be pathogenic on barley and oats; and *X. translucens* f. sp. *cerealis*, found naturally on wheat, was defined as being pathogenic on wheat, barley, oats, and rye (Hagborg 1942). Fang et al. (1950) agreed with Hagborg's description, except they observed no infection on oats. After observing a lack of infection on four varieties of oats by any isolate they tested, they proposed eliminating f. sp. *hordei-avenae* and expanded *X. translucens* f. sp. *hordei* to include those strains. Fang et al. (1950) also suggested that smooth brome and

quackgrass were the natural hosts of *X. translucens* f. sp. *cerealis* which they reported to be pathogenic on wheat, barley, rye, smooth brome, and quackgrass.

The term *forma specialis*, meant to describe an association with a particular host, was thought to be too precise for bacterial species having a distinct, but potentially wide host range (Young et al. 1978). In 1980, the infrasubspecific terminology would be replaced with the more inclusive term pathovar (pv.) to indicate host range, which might include multiple host species, versus host specificity, which implies an adaptation to a specific host (Dye et al. 1980; Young et al. 1978).

Bacteria from the genus *Xanthomonas* are capable of infecting both monocots and dicots, with at least 392 host species reported in the literature (Hayward 1993). Traditionally, new species were described each time an organism was discovered in a new host (Vauterin et al. 1995). Dye and Lelliott (1974) reduced the excessive naming by grouping the many previously described species into five *Xanthomonas* species. Following this nomenclature, the pathogens causing BLS were classified as *X. campestris*, which contained several pathovars differentiated by host range.

Xanthomonas campestris at one point contained over 140 pathovars (Vauterin et al. 1995, 1992). The excessive number of pathovars within this species essentially created the same problem that Dye and Lelliott (1974) had set out to solve, though at a different taxonomic level. Vauterin et al. (1995) proposed a reclassification of the genus *Xanthomonas* into twenty species based on protein electrophoresis, gas chromatography of fatty acids, and DNA-DNA hybridization. Strains isolated from small grains and grasses were identified as the same species. The taxonomic designation for BLS pathogens thus became *X. translucens* and strains with varying host ranges maintained

their previous pathovar descriptions (Vauterin et al. 1995). In accordance with the International Code of Nomenclature of Bacteria and the Standards for Naming Pathovars, the currently accepted nomenclature for BLS pathogens includes four pathovars of *X. translucens,* being pv. *cerealis*; pv. *secalis*; pv. *translucens*, and pv. *undulosa*, respectively (Table 1.1) (Bull et al. 2010; Hagborg 1942; Jones et al. 1917; Reddy et al. 1924; Smith et al. 1919). The formerly described pathovar *hordei*, which was reportedly pathogenic on barley, is no longer in use and these strains are considered to group with the strains within *X. translucens* pv. *translucens* (Duveiller et al. 1997).

1.6 Methods of Molecular and Genetic Characterization

The advancement and availability of molecular methods to aid in the identification and differentiation of the closely related taxa of *Xanthomonas* has played a large role in the reclassification of the genus. According to the recommendation of Wayne et al. (1987), DNA-DNA hybridization methods, with an arbitrary value of 70% DNA relatedness, has been used as the standard for differentiating species DNA-DNA hybridization and other methods analyzing protein content and methylated fatty acids have been used to distinguish *X. translucens* from other *Xanthomonas* species. These methods proved to be intensive, expensive, and not easily comparable across laboratories (Gevers et al. 2005; Young et al. 2008). Although useful at the species level these techniques could not separate the *X. translucens* pathovars from one another (Bragard et al. 1997; Vauterin et al. 1992). Sequencing of the 16S rDNA region has been proposed as a cost effective identification method for *X. translucens*, but it appears that it cannot distinguish *X. translucens* from the closely related species *X. albilineans* and

X. hyacinthi, species which also cause disease on monocots (Adhikari et al. 2012; Hauben et al. 1997).

Multilocus sequence analysis (MLSA) and multilocus sequence typing (MLST) use the concatenated sequence of multiple housekeeping genes to infer phylogenetic relationships and characterize organisms within a population, and these techniques have been used in multiple pathosystems to create phylogenies of closely related taxa, predict species and pathovar identification, and evaluate genetic diversity among strains (Bull et al. 2011; Croce et al. 2016; Curland et al. 2018; Maiden et al. 1998; Young et al. 2008). MLSA and MLST are a relatively inexpensive methods that provide results that are highly reproducible (Curland et al. 2018; Gevers et al. 2005; Maiden et al. 1998; Young et al. 2008). Sequence data generated from each locus can be deposited into public databases such as GenBank at the National Center for Biotechnology Information (http://ncbi.nlm.nih.gov) and the Plant Associated and Environmental Microbes Database (PAMDB) facilitating cross-study comparisons of strains (Almeida et al. 2010). Current studies on *X. translucens* have identified four housekeeping genes (*rpoD, dnaK, fyuA*, and *gyrB*) that can effectively distinguish *X. translucens* pathovars and be employed to evaluate the genetic diversity among strains (Curland et al. 2018; Young et al. 2008). Although MLSA and MLST are powerful molecular methods for aiding in the identification of *X. translucens* pathovars, by definition, pathovars are differentiated due to variation in their host range and thus the results of MLSA and MLST studies must be used in corroboration with *in planta* assays (Dye et al. 1980).

Loop mediated isothermal amplification (LAMP) is a PCR-based method that amplifies targeted DNA sequences of organisms that is conducted at a constant

temperature without the need for specialized equipment (Notomi et al. 2000). LAMP assays are a useful diagnostic tool that are highly sensitive, specific, rapid (60-70 min), and have the capability of being conducted in field or laboratory settings (Fischbach et al. 2015; Langlois et al. 2017). Assays have been developed to detect a variety of plant pathogens and is not specific to bacterial pathogens (Lang et al. 2014; Langlois et al. 2017; Villari et al. 2013).

LAMP assays have been developed to distinguish *X. translucens* from other *Xanthomonas* species as well as *X. translucens* pathovars that cause BLS on small grains from pathovars that cause bacterial wilt on grasses (Langlois et al. 2017). This tool can be used as a diagnostic strategy on a variety of materials. The assays can be conducted using genomic DNA, infected seed and plant tissue, or bacterial cultures (Langlois et al. 2017). Currently, LAMP assays used as a diagnostic tool using the primers developed to detect *X. translucens* pathovars have not been reportedly used but appear to be a valuable tool in the rapid identification of pathovars that cause BLS in small grains. Employing this tool could aid in monitoring pathogen populations in regions where BLS outbreaks are common and severe.

Chapter 2 . Pathovar identification and genetic diversity of *Xanthomonas translucens* strains isolated from weedy grasses and cultivated wild rice in Minnesota

2.1 Introduction

Bacterial leaf streak (BLS) of wheat (*Triticum aestivum* L.), caused by *Xanthomonas translucens* pv. *undulosa* (Smith et al. 1919) Vauterin et al. 1995, has been prevalent in Minnesota wheat fields for more than a decade with epidemics resulting in economic losses reported since 2005 (Curland et al. 2018). Symptoms in small grains typically begin as water-soaked lesions on the leaves progressing into greasy, translucent chlorotic and necrotic longitudinal streaks. As lesions coalesce the photosynthetic area is reduced, leaves die prematurely and yield is affected due to reduction in grain fill and kernel weight (Duveiller et al. 1997; Forster 1982; Smith et al. 1919). *X. translucens* is considered to be a seedborne pathogen although transmission rates are reported to be low (Duveiller et al. 1997; Tubajika et al. 1998). Crop debris, winter cereals, and perennial weeds may also serve as sources of primary inoculum (Duveiller et al. 1997; Thompson et al. 1989). Perennial grasses such as smooth brome (*Bromus inermis* Leyss.), quackgrass (*Elymus repens* (L.) Gould), and timothy (*Phleum pratense* L.) are also hosts of *X. translucens*, develop symptoms similar to those observed on small grain hosts, and are reported to facilitate overwintering (Boosalis 1952; Wallin 1946). Several other species of perennial grasses, such as *Hordeum, Festuca,* and *Poa* species, were identified as hosting epiphytic populations of the pathogen (Thompson et al. 1989). In contrast, there appears to be no published research on the role of annual poaceous weeds as alternative hosts for *X. translucens* pathovars. BLS has been reported on cultivated wild

rice (*Zizania palustris* L.) and quackgrass growing near wild rice fields in Minnesota (Bowden and Percich 1983; Kernkamp et al. 1976). Cultivated wild rice and wheat are produced in the same region of northern Minnesota, it is therefore plausible *X. translucens* pathovars affecting wheat might be present on wild rice.

There are currently nine recognized pathovars of *X. translucens*, four of which cause BLS on small grains (Table 1.1) (Bull et al. 2010; Hagborg 1942; Jones et al. 1917; Reddy et al. 1924; Smith et al. 1919). *X. translucens* pv. *undulosa* and *X. translucens* pv. *translucens* (Jones et al. 1917) Vauterin et al. 1995 are the two pathovars most commonly associated with BLS on wheat and barley in the Upper Great Plains, respectively (Adhikari et al. 2012; Curland et al. 2018). *X. translucens* pv. *undulosa* has a relatively wide host range and has been demonstrated to cause disease on wheat, barley (*Hordeum vulgare* L.), rye (*Secale cereale* L.), smooth brome, and quackgrass, while *X. translucens* pv. *translucens* has only been reported to cause symptoms on barley (Fang et al. 1950; Jones et al. 1917; Smith et al. 1919). *X. translucens* pv. *secalis* (Reddy et al. 1924) Vauterin et al. 1995 is reported to be pathogenic only on rye. *X. translucens* pv. *cerealis* (Hagborg 1942) Vauterin et al. 1995 has the same host range as *X. translucens* pv. *undulosa* but is most commonly found on smooth brome and quackgrass and more virulent on the grass hosts than the crop hosts (Bragard et al. 1997; Fang et al. 1950). Of the nine *X. translucens* pathovars, five pathovars; *X. translucens* pv. *arrhenatheri* (Egli and Schmidt 1982) Vauterin et al. 1995, *X. translucens* pv. *graminis* (Egli et al. 1975) Vauterin et al. 1995, *X. translucens* pv. *phlei* (Egli and Schmidt 1982) Vauterin et al. 1995, *X. translucens* pv. *phleipratensis* (Wallin and Reddy 1945) Vauterin et al. 1995, and *X. translucens* pv. *poae* (Egli and Schmidt 1982) Vauterin et al. 1995, are recognized

to cause bacterial wilt of grasses but they have not been reported to cause disease in small grains.

Multilocus sequence analysis (MLSA) and multilocus sequence typing (MLST) of a small number of housekeeping genes have been used to differentiate closely related bacterial species, predict pathovar designation, and assess genetic diversity among closely related strains (Bull et al. 2011; Croce et al. 2016; Curland et al. 2018; Maiden et al. 1998; Young et al. 2008). These molecular analyses are highly reproducible and as the sequence data can be utilized to compare bacterial strains across studies, facilitating studies of bacterial pathogens that were not possible using the classical morphological techniques (Curland et al. 2018; Maiden et al. 1998; Young et al. 2008). Previous MLSA studies using four housekeeping genes (*rpoD*, *dnaK*, *fyuA*, and *gyrB*) have been used to differentiate *Xanthomonas* spp. from one another as well as to predict pathovars of *X. translucens* (Curland et al. 2018; Young et al. 2008). MLSA is useful to predict pathovar designation, however, by definition *in planta* assays would still be required to confirm pathovar identification (Dye et al. 1980).

Loop mediated isothermal amplification (LAMP) is a PCR-based method that amplifies targeted DNA sequences of organisms that is conducted at a constant temperature without the need for specialized equipment (Notomi et al. 2000). LAMP assays are a useful diagnostic tool that are highly sensitive, specific, rapid (60-70 min), and can be completed in field or laboratory settings (Fischbach et al. 2015; Langlois et al. 2017). LAMP assays have been developed to detect all pathovars of *X. translucens* as well as distinguish *X. translucens* pathovars that cause BLS on small grains from pathovars that cause bacterial wilt on grasses (Langlois et al. 2017). However, available

LAMP assays cannot distinguish *X. translucens* pv. *translucens* from *X. translucens* pv. *undulosa*. While, MLSA provides a more specific prediction of pathovar identity it is more time consuming, requires specialized equipment, and is more expensive.

The objectives of this study were to 1) identify and evaluate the genetic diversity of *X. translucens* pathovars present on weedy grasses and cultivated wild rice and 2) evaluate LAMP assays, previously developed for *X. translucens* pathovars from small grains, for use in identifying a diverse group of *X. translucens* strains. The findings of this study can provide wheat growers and breeders with information for the development of BLS management strategies.

2.2 Materials and Methods

2.2.1 Plant samples and bacteria strain isolation

A total of 157 plant leaves were collected between July and August in 2016 and 2017 from 18 different field sites in seven Minnesota counties (Table 2.1). Leaf samples came from individual plants growing in and around fields exhibiting typical BLS symptoms. The plant samples included samples of nine species of weedy grasses, in addition to barley, wheat, and cultivated wild rice (Table 2.2). Leaf samples were stored in sealed plastic bags at 4°C until isolations were performed.

Bacteria were isolated from leaf tissue within 30 days of the collection date. Approximately 1 cm^2 of leaf tissue was chopped into small pieces, placed into a 1.7 ml microcentrifuge tube containing 1 ml of 0.85% NaCl solution, and placed on a rocker for 20 minutes. The resulting suspensions were serially diluted to 10^{-4} and 10^{-6} and 100 µl of

each dilution was plated onto Wilbrink's agar (WBA) then incubated at 27°C for 3 to 5 days. Single colonies with the shiny, bright-yellow appearance characteristic of *Xanthomonas* spp. were subcultured to obtain pure cultures. One to five colonies per sample were selected and stored at –80°C in nutrient broth with yeast extract (NBY) containing 10% glycerol.

2.2.2 Reference strains

Fourteen *X. translucens* strains were chosen to serve as reference strains for *in planta* and/or molecular studies (Table 2.3). The species type strain, *X. translucens* pv. *translucens* (LMG 876^{T}), and the pathotype strains of *X. translucens* pv. *cerealis* (LMG 679^{PT}), *X. translucens* pv. *graminis* (LMG 726^{PT}), *X. translucens* pv. *secalis* (LMG 892^{PT}), and *X. translucens* pv. *undulosa* (LMG 883^{PT}), obtained from the Belgian Co-ordinated Collections of Micro-organisms (BCCM/LMG), were used in the molecular analyses. *Xanthomonas translucens* pv. *graminis* is the taxa most distantly related to the *X. translucens* pathovars that cause disease on small grain crops, therefore the pathovar strain LMG 726^{PT} was chosen to serve as an outgroup strain in the analyses (Curland et al. 2018; Young et al. 2008).

Sequences of five strains representing *X. translucens* pv. *translucens* MLSA clades Xtt A (CIX43), Xtt B (CIX84), and Xtt C (CIX41 and CIX95) and *X. translucens* pv. *undulosa* (CIX40) as reported by Curland et al. (2018) were used in molecular analyses. Two of these strains, CIX95 and CIX40, also served as the reference strains for *X. translucens* pv. *translucens* and *X. translucens* pv. *undulosa*, respectively in the *in*

planta assays. In addition, four strains characterized by Bowden and Percich (1983), isolated from cultivated wild rice (Xt-8 and Xt-22) and quackgrass (Xt-2 and Xt-25), served as reference strains in the molecular analyses of four strains from wild rice and six strains from quackgrass collected in this study that were isolated from samples originating in and around cultivated wild rice fields.

2.2.3 DNA extraction, amplification, and sequencing

Strains were recovered from storage and single colonies were subsequently grown as bacterial lawns on WBA at 27°C. A sterile swab of these cultures was used to transfer a dab of a three-day-old pure culture into 1 ml of Tris EDTA (pH 8) buffer. The resulting cell suspension was stored at –20°C until DNA extractions were performed. Genomic DNA was extracted using the DNeasy Blood & Tissue Kit following the manufacture's protocol for Gram-negative bacteria (Qiagen, Germantown, MD). Extracted DNA was stored in the kit's elution buffer (buffer AE) at –20°C.

The 16S rDNA region was sequenced using primers 27F (5′-AGAGTTTGATCATGGCTCAG-3′) and 1492R (5′-GGTACCTTGTTACGACTT-3′) (Lane 1991) and thus predicted 87 strains to be *X. translucens* (strains used in this study) and 47 strains to be other *Xanthomonas* species (Table 2.2) (Adhikari et al. 2012; Hauben et al. 1997).

Four housekeeping genes (*rpoD*, *dnaK*, *fyuA*, and *gyrB*) were amplified and sequenced for MLSA and MLST, with the gene selection based on previous studies (Curland et al. 2018; Young et al. 2008; Zacaroni et al. 2012). Each 50 µl reaction, used

for DNA amplification, was set-up using the HotStarTaq Master Mix Kit (Qiagen, Germantown, MD) and contained 30.75 µl of sterile water, 5 µl coral load buffer, 10 µl Q-solution, 1 µl dNTPs, 1 µl each of forward (F) and reverse (R) primers, 0.25 µl HotStarTaq, and 1µl DNA template. The primer concentrations for each housekeeping gene were as follows: *rpoD*, 10 µM F and 20 µM R; *dnaK*, 10 µM F and 10 µM R; *fyuA*, 20 µM F and 10 µM R; and *gyrB*, 10 µM F and 10 µM R. PCR amplifications were performed with an initial denaturation at 95°C for 15 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, extension at 72°C for 1 min, followed by a final extension at 72°C for 10 min for *rpoD*, *dnaK*, and *fyuA*. The cycle conditions for *gyrB* were the same as described for the other three genes, except for the annealing temperature, which was 54°C (Curland et al. 2018). Gel electrophoresis was used to visualize PCR products on a 1% agarose gel stained with ethidium bromide in TBE buffer. Amplified products were sent to MCLAB (South San Francisco, CA) for PCR purification and Sanger sequencing.

2.2.4 Multilocus sequence analysis

Sequences were assembled, trimmed, and aligned using CLC Main Workbench 7 (Qiagen, Germantown, MD). Sequences were trimmed as in previous studies, so as to provide consistency for the comparison of strain sequence data across studies (Curland et al. 2018; Zacaroni et al. 2012). Gene fragments of *rpoD*, *dnaK*, *fyuA*, and *gyrB* were trimmed to 674, 762, 522, and 687 nucleotides, respectively, resulting in a concatenated length of 2645 nucleotides. Model testing in CLC Workbench 7 identified the general

time reversible model (GTR+G+T) as the best fit and so this model was used in tree construction. Bayesian MCMC analysis was performed using the program Bayesian Evolutionary Analysis Sampling Trees (BEAST v1.8.4) (Drummond et al. 2012). The analysis assumed a strict molecular clock and 500 million generations. The programs Tracer v1.6 (Rambaut et al. 2014) and FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree) were used to analyze and visualize the output, respectively. CLC Workbench 7 was also used for final tree edits and to facilitate the inclusion of metadata (host of origin and character state) in the figures.

2.2.5 Multilocus sequence typing

Sequence variation was analyzed using DnaSP v6.10.04 (Rozas et al. 2017). Allele numbers were given to unique sequences for each of the four loci. Sequence type (ST) numbers were assigned to each unique concatenated sequence. The numbers assigned were congruent with the work of Curland et al. (2018) so that ST data could be compared across studies. When a strain had an allele or concatenated sequence not previously described it was assigned the next available consecutive number.

Clonal complexes, along with their predicted founding ST, were identified using goeBURST and visualized as a minimum spanning tree created with goeBURST Full MST in PHYLOViZ 2.0 (Nascimento et al. 2017). The ‘single locus variant’ default setting was used, creating clonal complexes of strains that shared identical alleles at three of the four loci examined (Feil et al. 2004; Nascimento et al. 2017). Host of origin was added to the resulting figures as metadata.

2.2.6 Greenhouse seedling assays

Twenty strains isolated in 2016, predicted to be *X. translucens* by 16S rDNA sequencing, were tested for pathogenicity and virulence on wheat (cv. RB07) and barley (cv. Quest) seedlings in two trials conducted in May 2017 and in October 2017, respectively. Similarly, two trials were conducted in October 2017 and in November 2017 on 31 *X. translucens* strains isolated in 2017 that were also predicted to be *X. translucens* from their 16S rDNA sequence. For each of the four trials, *X. translucens* pv. *undulosa* strain CIX40 and *X. translucens* pv. *translucens* strain CIX95, were selected based on previously published research to serve as positive controls (Curland et al. 2018). A saline solution (0.85% NaCl) was used as the mock-inoculated treatment and served as a negative control.

The greenhouse assays were conducted in the University of Minnesota Plant Growth Facility West, St. Paul, MN. All trials used a randomized complete block design with three replicates, with each strain tested on each of two host crops (wheat and barley). Wheat and barley seedlings were grown in plastic pots (6.4 cm diameter × 25.4 cm deep; Deepots, Stuewe & Sons, Inc., Tangent, OR) with one seed planted per pot and pots filled with a mixture of 60% sterile soil and 40% Sunshine Mix #8/Farfard-2 soilless media (Sun Gro Horticulture, Quincy, MI). Daytime greenhouse temperatures were set between 18 and 20°C and nighttime temperatures were set between 15 and 17°C. Fifteen hours of supplementary light provided by 400 W high-pressure sodium lamps. The plants were watered as needed, either daily or every other day. Each pot received 2.5 g of a slow release fertilizer (14-14-14, N-P-K; Osmocote Classic, Everris NA, Inc., Dublin,

OH) and 0.5 g of 1% imidacloprid granular insecticide (Marathon, OHP, Inc., Mainland, PA) one week after planting.

Bacterial cells from freezer stocks were grown for three days at 27°C on WBA and subcultured as bacterial lawns on WBA. Cells from the lawns were suspended in a 0.85% NaCl solution to obtain approximately 10^7 CFU/ml (OD_{540} = 0.01). Seedling leaf tissues were infiltrated with the bacterial suspensions at the 2-4 leaf stage using a blunt end syringe with an orifice 2 mm in diameter. The second and third leaves on each plant were inoculated at a point along the midvein, approximately 10 cm from the leaf sheath, until inoculum had visibly infiltrated into the leaf blade about 1 cm from the inoculation point. Each inoculated leaf was marked at the tip of the leaf blade, using a black permanent marker, to identify the inoculated leaves. Character states (water-soaking, chlorosis, or no response) were recorded 5-6 days post inoculation (dpi) and used to determine pathogenicity. Lesion length (cm) was measured 9-10 dpi and used to assess virulence.

2.2.7 Field assessments

Field trials to assess strain virulence and host response were conducted at the University of Minnesota's Agricultural Experimental Station at the Saint Paul campus during the growing seasons of 2017 and 2018. Field experiments were a split-plot treatment arrangement, randomized complete block design with four replications, where bacterial strains were the whole plot treatment and host crops were the sub-plot treatment. Eight *X. translucens* strains from weedy grasses were evaluated along with previously described strains CIX40 (*X. translucens* pv. *undulosa*) and CIX95 (*X. translucens* pv.

translucens) (Curland et al. 2018). In addition to the ten strains a mock-inoculated treatment, using a 0.85% NaCl solution, served as a negative control. Each of the 11 treatments were inoculated onto 2 m single row plots of the wheat cultivars Blade and RB07, the barley cultivar Quest, and the oat (*Avena sativa* L.) cultivar Goliath. RB07 and Quest were considered to be moderately susceptible to BLS, while Blade was listed as resistant (Minnesota Agricultural Experiment Station 2012, 2017). Although BLS has not been reported as a disease of oat in Minnesota, oat was included in the experiments as three *X. translucens* strains (CIX288, CIX290, and CIX297) originated from wild oat. The popular oat cultivar Goliath was selected for this experiment.

Inoculum of the ten strains tested was prepared from glycerol freezer stocks, with cultures grown on WBA for four days at 27°C and subsequently subcultured as bacterial lawns on WBA. Subcultures were achieved by streaking nine zig-zag streaks across the plate, using a sterile swab, and cells were grown for three days at 27°C. One plate per culture was suspended in 3 liters of 0.85% NaCl solution resulting in an approximate cell concentration of 10^8 CFU/ml. Each 2 m plot was sprayed for six seconds using a CO_2-powered backpack sprayer, operating at a pressure of 276 kPa, with an output of ca. 10 ml/s. Plants were inoculated at the 'tillering' growth stage (Feekes 4-5) in 2017 and at 'early boot' (Feekes 10-10.1) in 2018.

Disease severity of individual plots were assessed using the adapted Saari-Prescott double digit (00-99) scale (Saari and Prescott 1975) as described by Kandel et al. (2012). In this scale, the first digit (D_1) represents the vertical disease progression in the plant canopy on a 0 to 9 scale, where 0 indicates no infection and 9 indicates disease progression to the top of the plants in the plot. The second digit (D_2) represents BLS

severity based on the percentage leaf area of the plot that is symptomatic based on a 0 to 9 scale, where 0 indicates no infection and 9 indicates 90% symptomatic leaf area of the plot. Disease scores were taken at 10, 13, and 17 dpi in 2017 and at 7 and 10 dpi in 2018. Percent disease severity [$(D_1/9) \times (D_2/9) \times 100$] for each data collection point was used to calculate the area under the disease progress curve (AUDPC) for each year (Duveiller et al. 2005; Kandel et al. 2012).

2.2.8 Statistical analyses of greenhouse and field data

Greenhouse virulence data were analyzed using R (version 3.5.3) package 'agricolae' (de Mendiburu 2019). The mean lesion length for the two inoculated leaves per plant was calculated and the mean used in further analyses, i.e. individual leaf lesion lengths were not treated as subsamples. The lesion lengths in all experiments were log transformed to meet the assumptions for two-way analysis of variance (ANOVA) where host crop and strain were independent factors. Log transformed lesion lengths were back-transformed for presentation and visualization of the data. Tukey's honestly significant difference was used for mean separation tests. A Spearman's rank order correlation test was used to compare the two trials examining the 2016 strain collection and the two trials examining the 2017 strain collection. As the two strain collections had little overlap, no analysis was undertaken to compare trials across the two strain collections.

Field data were analyzed using R (version 3.5.3) package 'lmerTest' (Kuznetsova et al. 2017). To minimize the effect of background disease on plot scores, the data were adjusted based on the percent disease severity for mock-inoculated treatments of each

host crop within each replication. Thus, percent disease severity in the mock-inoculated treatments on wheat, barley, and oat cultivars were subtracted from the inoculated host crop of the same cultivar within the same replication. Means of the adjusted percent disease severity across the four replications was used to calculate the AUDPC for each host crop × strain treatment combination. ANOVA for the variable AUDPC was performed using a mixed model, where replication was considered a random effect and strain and host crop were independent factors. Tukey's honestly significant difference was used for mean separation tests.

2.2.9 Loop-mediated isothermal amplification (LAMP) assays

Genomic DNA of 33 *Xanthomonas* strains, representing 24 different STs (Appendix Table 2.1), were used in LAMP detection assays to evaluate the efficacy of a subset of diagnostic primer sets (*gyrB*-Xt, Xt-CLS, and Xt-Cerealis) developed by Langlois et al. (2017) to detect *X. translucens* pathovars with varying STs. The three primer sets were selected based on their ability to identify *X. translucens* (*gyrB*-Xt) and pathovars known to be pathogenic on small grain crops (Xt-CLS and Xt-Cerealis) (Langlois et al. 2017). Nineteen of the 33 tested strains were *X. translucens* strains isolated in this study. Seven previously described strains were also included to provide representative strains of the *X. translucens* pv. *translucens* MLSA-identified clades Xtt A, Xtt B, and Xtt C (Curland et al. 2018). The species type strain, *X. translucens* pv. *translucens* (LMG 876^{T}), and the pathotype strains of *X. translucens* pv. *cerealis* (LMG 679^{PT}), *X. translucens* pv. *graminis* (LMG 726^{PT}), *X. translucens* pv. *poae* (LMG 728^{PT}), *X. translucens* pv. *secalis* (LMG 892^{PT}), and *X. translucens* pv. *undulosa* (LMG 883^{PT})

were also included. One strain, CIX286, predicted to be a *Xanthomonas* species other than *X. translucens* based on 16S rDNA sequence and non-pathogenic responses on wheat and barley in a preliminary greenhouse assay (data not shown), along with a sterile water sample served as negative and experimental controls, respectively.

Reaction mixtures for each of the three primer sets included 7.2 µl Isothermal Master Mix (OptiGene, Sussex, United Kingdom), 640 nM FIP/BIP, 64 nM F3/B3, and 1 µl DNA template per 12 µl reaction. The reaction mixture for the Xt-Cerealis primer set was modified from the protocol described by Langlois et al. (2017), as only half the concentration of the FIP, BIP, F3, and B3 primers used in the Langlois et al. (2017) study were used. Reactions were carried out and visualized using the Genie III (OptiGene, Sussex, United Kingdom) instrument with an incubation period of 70 min at 65°C with a subsequent melt curve analysis in 0.5°C increments from 60 to 95°C (Langlois et al. 2017). Each LAMP assay was replicated.

2.3 Results

2.3.1 Multilocus sequence analysis

Amplification was successful for all four loci (*rpoD*, *dnaK*, *fyuA*, and *gyrB*) for the strains sequenced in this study. The phylogeny of the concatenated sequences created from the Bayesian analysis showed five distinct clades, three *X. translucens* pv. *translucens* clades A (Xtt A), B (Xtt B), and C (Xtt C), *X. translucens* pv. *undulosa* (Xtu), and *X. translucens* pv. *cerealis* (Xtc), with *X. translucens* pv. *graminis* pathotype LMG 726^{PT} as the outgroup (Fig. 2.1 and Table 2.4). Most (83.2%) of the strains fell

within the Xtu clade. The five reference strains from Curland et al. (2018) (CIX40, CIX41, CIX43, CIX84, and CIX95) belonged to the same clades as described in their MLSA study and the topology of the tree created in this study was similarly consistent to their findings, showing three distinct clades of *X. translucens* pv. *translucens* with Xtt A nested within the Xtu clade. The tree topology in the current study showed more variation, depicting some sub-structure within the Xtu clade than was previously reported by Curland et al. (2018) which described the Xtu clade as monophyletic.

The type strain for *X. translucens* pv. *translucens* (LMG 876^{T}) and reference strain CIX43 grouped with Xtt A, reference strain CIX84 grouped with Xtt B, and reference strains CIX41 and CIX95 grouped with Xtt C. All strains isolated in this study that originated on barley fell into one of the three Xtt clades: Xtt A, Xtt B, or Xtt C. The *X. translucens* pv. *undulosa* (Xtu) clade included the pathotype strains for *X. translucens* pv. *undulosa* (LMG 892^{PT}) and *X. translucens* pv. *secalis* (LMG 883^{PT}) as well as reference strain CIX40. The four reference strains isolated from wild rice and quackgrass (Xt-2, Xt-8, Xt-22, and Xt-25) and described as *X. campestris* pv. *cerealis* by Bowden and Percich (1983) also fell into the Xtu clade. The Xtu clade thus contained all strains examined in this study that originated from wheat, wild rice and all the weedy grass species, except smooth brome. The Xtc clade contained the pathotype strain for *X. translucens* pv. *cerealis* (LMG 679^{PT}) and all four strains originating from smooth brome.

All 51 strains tested *in planta*, except CIX95, showed water-soaking on both barley and wheat. The character states of these strains were the same as those of the *X. translucens* pv. *undulosa* reference strain CIX40 and all fell within the Xtu clade

except the strains originating from smooth brome. The strains isolated from smooth brome were not distinguishable from strains isolated from other weedy grasses based on their character states *in planta,* although they showed a distinct separation in the MLSA phylogeny. CIX95 was the only strain from the Xtt clade tested *in planta*, and this strain had a unique character state distinguished from the other strains tested as it exhibited water-soaking only on barley.

2.3.2 Multilocus sequence typing

Twenty STs were identified based on their unique concatenated sequences from the 101 strains analyzed by MLST. Five clonal complexes were evident in the minimum spanning tree created in PHYLOViZ (Fig. 2.2). Each complex was determined by single locus variants and members of each group were predicted to have descended from the same founding sequence type. The *X. translucens* pv. *graminis* pathotype, ST 63, was the only strain in its clonal complex and was used as an outgroup strain in the analysis. Clonal complex Xtc included three STs all originating from smooth brome with ST 60 as the predicted founder ST. The Xtc complex also included the pathotype strain of *X. translucens* pv. *cerealis* (LMG 679). Two clonal complexes were identified in the Xtt group, each containing two STs, and all strains originated from barley. The first of the clonal complexes represented clade Xtt A, with the predicted founder being ST 15. Clade Xtt A also included the type strain for *X. translucens* pv. *translucens* (LMG 876). The second clonal complex in the Xtt group included within it both the Xtt B and Xtt C clades. Twelve STs originating from eight different host species (foxtail barley, green foxtail, perennial ryegrass, quackgrass, rye, wheat, wild rice, and wild oat) comprised the

clonal complex for the Xtu clade. The predicted founder ST for the Xtu clade was ST 36 which included the pathotype strain for *X. translucens* pv. *undulosa* (LMG 892). The Xtu clade also had the most diversity of the strains included in the study with respect to host of origin, including strains originating from six host species.

2.3.3 Greenhouse seedling assays

The character states observed in the greenhouse seedling assays were used to determine pathogenic versus non-pathogenic host responses. The character state described as water-soaking was considered a pathogenic response, while the character state of chlorosis, restricted to the area of infiltration, was considered a non-host reaction (Appendix Fig. 2.2). Strain CIX95 of *X. translucens* pv. *translucens* produced non-expanding chlorotic lesions on wheat and water-soaked lesions on barley, as was reported previously (Curland et al. 2018), and was therefore considered to be pathogenic on barley and not wheat. The character states of the 51 strains tested (ex. the 2016 and 2017 strain collections) were identical to the reference strain of *X. translucens* pv. *undulosa* (CIX40). These strains exhibited water-soaked lesions on both wheat and barley. Based on these character states, it was determined that CIX40 and the 51 strains from the 2016 and 2017 strain collections were pathogenic on both wheat and barley. No symptoms developed in the mock inoculated treatment.

Mean lesion length across strains was found to be significantly different ($Prob>F < 0.001$) between the two trials testing the 2016 strain collection and also the two trials testing the 2017 strain collection. Therefore, each trial was analyzed individually. The ANOVA identified strain, host crop, and the strain × host crop

interaction as significant (Prob>F < 0.001 for all factors) for the first trial examining the 2016 strain collection. Strain and host crop were significant (Prob>F < 0.001 for both factors) in the second trial examining the 2016 stain collection, but the interaction of strain × host crop was not (Prob>F = 0.29). In both trials examining the 2017 strain collection, the ANOVA identified strain, host crop, and a strain × host crop interaction as significant (Prob>F < 0.001 for all factors). Strains were statistically more virulent on wheat than barley in the first trial that examined the 2016 strain collection, though in the second trial that examined the 2016 stain collection strains were more virulent on barley than wheat (Table 2.5). In both trials that examined the 2017 collection the strains were more virulent on barley than on wheat (Table 2.5).

The Spearman's rank correlation tests resulted in statistically significant positive correlations between strains from the 2016 collection in both trials on wheat ($r_s(21) = 0.60$, $P = 0.003$) and barley ($r_s(21) = 0.52$, $P = 0.01$). Significant positive correlations were also identified between strains from the 2017 collection in both trials on wheat ($r_s(32) = 0.77$, $P < 0.001$) and barley ($r_s(32) = 0.49$, $P = 0.004$). Although the lesion lengths of the strains infiltrated on wheat and barley differed among trials, virulence rankings were similar between the two trials that examined the 2016 strain collection and the two trials that examined the 2017 strain collection. The data from the two trials that examined the 2016 strain collection were combined for visualization, as was the data from the two trials examining the 2017 strain collection (Figures 2.3 and 2.4).

Virulence varied among the strains in the 2016 strain collection and was dependent on the host crop with a range of back-transformed mean lesion lengths from

1.0 cm to 5.6 cm on wheat and 1.6 cm to 6.9 cm on barley (Fig. 2.3). Similarly, virulence also varied among the strains in the 2017 strain collection and was dependent on the host crop with a range of back-transformed mean lesion lengths from 0.8 cm to 2.8 cm on wheat and 1.4 cm to 3.8 cm on barley (Fig. 2.4).

2.3.4 Field assessments

BLS symptoms developed on the wheat cultivars Blade and RB07, and on the barley cultivar Quest in both experimental years. No disease development was observed on the oat cultivar Goliath in either year of the study. Background infections were observed in the mock-inoculated barley plots in 2017 and in both the mock-inoculated wheat and barley plots in 2018, therefore adjusted disease scores were used for analyses. Disease severity, as measured by AUDPC, could not be compared between years because environmental conditions did not allow for consistency in the timing of inoculation and evaluation with respect to the crop growth stage or the number of days after planting or the number of days post inoculation. In the 2017 field trials, the ANOVA identified strain, host crop, and a strain × host crop interaction as significant ($Prob>F < 0.001$ for all factors). All strains tested were more virulent on wheat than on barley, except strain CIX95 (*X. translucens* pv. *translucens*) that caused disease only on barley (Fig. 2.5). In the 2018 field trial, the ANOVA identified strain and host crop ($Prob>F < 0.001$ for both factors) as well as a strain × host crop interaction as significant ($Prob>F = 0.002$). Similar to the 2017 field trail, more disease developed on the wheat cultivars than on the barley for all strains tested in 2018, except CIX95. Some disease was observed in the wheat plots inoculated with CIX95 in 2018 and the disease was still significant even after

the disease severity scores were adjusted for background infections (Fig. 2.6). In both experimental years, the AUDPC for BLS in the two wheat cultivars Blade and RB07 did not significantly differ from each other, but both were significantly different from the barley cultivar Quest based on Tukey's HSD at $P < 0.05$. The responses of wheat and barley inoculated with the eight strains isolated from weedy grasses resembled the responses to strain CIX40 (*X. translucens* pv. *undulosa*).

2.3.5 Loop-mediated isothermal amplification (LAMP) assays

The initial LAMP assays used three different primer sets and included the type and pathotype strains, *X. translucens* pv. *translucens* (LMG 876^{T}), *X. translucens* pv. *cerealis* (LMG 679^{PT}), *X. translucens* pv. *graminis* (LMG 726^{PT}), *X. translucens* pv. *poae* (LMG 728^{PT}), *X. translucens* pv. *secalis* (LMG 883^{PT}), and *X. translucens* pv. *undulosa* (LMG 892^{PT}), along with *Xanthomonas* sp. (CIX286) and a sterile water were included as a negative reference strain and control, respectively. Primer set *gyrB*-Xt, designed to identify all *X. translucens* pathovars, amplified the target DNA of all six type and pathotype strains (Appendix Fig. 2.3). Primer set Xt-CLS, designed to identify *X. translucens* pv. *translucens*, *X. translucens* pv. *secalis*, and *X. translucens* pv. *undulosa*, successfully amplified the target DNA thus identifying the three pathovars associated with these type strains (LMG 876^{T}, LMG 883^{PT}, and LMG 892^{PT}, respectively) (Appendix Fig. 2.4). Primer set Xt-Cerealis was designed to be specific to *X. translucens* pv. *cerealis* and amplified the target DNA of only the *X. translucens* pv. *cerealis* pathotype strain (LMG 679^{PT}) (Appendix Fig. 2.5). The *Xanthomonas* sp. (CIX286) and sterile water negative controls did not amplify using any of the three

primer sets. Based on these initial results, all subsequent assays included LMG 892^{PT} and LMG 679^{PT} as positive controls and a sterile water sample as a negative control. The remaining 26 *X. translucens* strains tested, including all 24 STs identified by MLST, were successfully amplified using the *gyrB*-Xt primers. The Xt-CLS primers did not amplify any of the five *X. translucens* pv. *cerealis* strains. The Xt-CLS primer set amplified all *X. translucens* pv. *undulosa* and *X. translucens* pv. *translucens* strains except three strains from the MLSA clade Xtt A, all of which had been identified as ST 24 in the MLST analysis. The Xt-Cerealis primers amplified all *X. translucens* pv. *cerealis* strains.

2.4 Discussion

In our study, *X. translucens* pv. *undulosa* was the most common xanthomonad isolated from weedy grasses and cultivated wild rice in Minnesota. All strains isolated from wheat, wild rice, and grasses (except for smooth brome) fell into the Xtu clade based on the MLSA using four housekeeping genes (*rpoD*, *dnaK*, *fyuA*, and *gyrB*). *In planta* character states of water-soaking on wheat and barley supported the pathovar designation of *X. translucens* pv. *undulosa* as described by Smith et al. (1919) for the strains in the Xtu clade. Strains isolated from smooth brome had similar character states to all the other strains isolated from grasses, showing water-soaking on both wheat and barley, but the smooth brome strains grouped into a distinct MLSA clade (Xtc), indicating a phylogenetic divergence of these strains from other *X. translucens* pathovars. MLSA was successful in predicting the *X. translucens* strains isolated from weedy grasses and wild rice to the pathovar level, and the MLSA predictions were confirmed by the *in planta* host response assays on wheat and barley.

Previous MLSA studies using the same four loci have also demonstrated a clear separation of the pathovars *X. translucens* pv. *undulosa* and *X. translucens* pv. *cerealis* (Curland et al. 2018; Young et al. 2008). *X. translucens* pv. *cerealis* was defined by its broad host range, including smooth brome and quackgrass, and by greater virulence on these hosts than *X. translucens* pv. *undulosa* (Fang et al. 1950). The *X. translucens* pv. *cerealis* strains reported in our study, that originated from smooth brome, had shorter mean lesion lengths on wheat in greenhouse assays compared to strains determined to be *X. translucens* pv. *undulosa*. However, virulence was not assessed on smooth brome or quackgrass in greenhouse or field assays in our study, limiting the ability to distinguish *X. translucens* pv. *undulosa* strains from *X. translucens* pv. *cerealis* strains *in planta*.

All strains identified in the MLSA clade Xtu of our study fell within the same clonal complex comprised of 12 different sequence types (Figures 2.1 and 2.2). The substructure in the Xtu clade evident from the MLSA (Fig 2.1) indicates variation within *X. translucens* pv. *undulosa*. The variation within the Xtu clade has not been reported in previous MLSA phylogenies of Minnesota strains isolated from wheat (Curland et al. 2018). As the current study identifies strains present on a broader host range than previous studies, including strains from weedy grass species and wild rice, the broader host range examined may have been reflected in the greater genetic variation observed in our study.

The founding sequence type for the *X. translucens* pv. *undulosa* clonal complex in our MLST study (ST 36) was congruent with that of a previous report (Curland et al. 2018). ST 36 was isolated from six different hosts of origin including wheat and perennial and annual grass species. The fact that ST 36 has been identified across time,

across locations, and now on multiple hosts provides further evidence that this genotype is adapted to the Upper Great Plains, including Minnesota. Our findings identified four additional STs (ST 29, ST 30, ST 34, and ST 38) that were isolated from grasses and wild rice which have also been associated with recent BLS outbreaks on wheat in the Upper Great Plains (Curland et al. 2018). This finding suggests that the populations found on weedy grasses and wild rice are associated with BLS on wheat and are relatively stable in time and space. Identifying the conserved STs in the population provides wheat breeders with information to select appropriate strains of *X. translucens* pv. *undulosa* to facilitate the development of varieties with improved resistance to BLS in the region.

X. translucens was isolated from both annual and perennial grasses in and around Minnesota wheat fields. *X. translucens* has previously been demonstrated to overwinter in the perennial hosts smooth brome and quackgrass in the Upper Great Plains (Boosalis 1952; Wallin 1946). Smooth brome and quackgrass are common grasses found growing around field margins and are among the grass species that are the first to grow in the spring, serving as a potential overwintering host and reservoir of primary inoculum that may spread to small grains (Boosalis 1952; Wallin 1946).

Of the perennial grasses examined, quackgrass appears the most likely to play a role in BLS epidemiology. Quackgrass was present in and sampled from most (16/18) of the field locations in this study and was consequently the most heavily represented host, with 28 samples collected. *X. translucens* pv. *undulosa* was isolated from most of these quackgrass samples and often multiple strains of *X. translucens* pv. *undulosa* were isolated from a single plant sample. In contrast, foxtail barley, also a perennial grass, was sampled almost as often as quackgrass (22 foxtail barley and 28 quackgrass samples),

however only four *X. translucens* pv. *undulosa* strains were isolated from the foxtail barley samples collected in this study. Seven *X. translucens* pv. *undulosa* strains were isolated from four perennial ryegrass samples, however as these perennial ryegrass samples all originated from a single field, they are insufficient to draw conclusions about the distribution of this pathovar on this host. *X. translucens* pv. *cerealis* was the only pathovar identified on smooth brome and although this pathovar is capable of causing disease on small grains, it is not thought to be a pathogen of major concern (Bragard et al. 1997; Curland et al. 2018; Fang et al. 1950).

The annual grasses identified in and around wheat fields in this study were wild oat and green foxtail. Eleven *X. translucens* pv. *undulosa* strains were isolated from wild oat. These strains were found in both 2016 and 2017 at six of eight field locations sampled. Five *X. translucens* pv. *undulosa* strains were isolated from green foxtail, these all originated from one of the five field locations sampled. Sampling of these annual species may be insufficient to draw conclusions on the importance of the pathogen populations present on these hosts in our study, although it is evident that the annual grasses have the potential to harbor *X. translucens* pv. *undulosa*. Our study demonstrates that *X. translucens* pv. *undulosa* is present on both perennial and annual grasses and so these hosts should be considered as potential reservoirs for the pathogen, contributing to survival and the population size throughout the growing season.

There was considerable variation in the number of fields sampled and the number of plants sampled per field in this study. We recognize that the strains obtained in this study are inherently biased by our sampling protocol and thus we would not expect that the strains we collected to be fully representative of the overall population. A strategic

sampling system could be employed to reduce bias of overrepresentation of one host species and further studies would be needed to identify all the weedy grasses that could harbor *X. translucens* pv. *undulosa*. It should also be noted that alternative hosts in association with wheat fields were the focus of this study where BLS is typically caused by *X. translucens* pv. *undulosa*. Potential reservoirs and pathovars present may be very different in and around barley fields where BLS is typically caused by *X. translucens* pv. *translucens*.

BLS has been reported on cultivated wild rice and neighboring quackgrass in Minnesota, but little research has focused on BLS in this host or on the epidemiology of this disease (Bowden and Percich 1983; Kernkamp et al. 1976). Four strains isolated by Bowden and Percich (1983), described as *X. campestris* pv. *cerealis* by them, were obtained and included in the molecular analyses in this study. Although the methods used to identify the two strains that originated from quackgrass (Xt-2 and Xt-25) were not clearly described by Bowden and Percich (1983), the pathovar designation of *X. campestris* pv. cerealis for these two strains appears to have been assigned because they were isolated from quackgrass (Dye et al. 1980; Fang et al. 1950). The strains Xt-2 and Xt-25 were used as reference strains in the study conducted by Bowden and Percich (1983) and they reported that the symptom profiles of the two strains originating from wild rice (Xt-8 and Xt-22) were identical to the quackgrass strains and identified as *X. campestris* pv. *cerealis* in their host range study. Bowden and Percich (1983) reported that Xt-25 and Xt-8 exhibited water-soaking on wheat, quackgrass, barley, and wild rice, while Xt-2 and Xt-22 exhibited water-soaking on wheat, quackgrass, and wild rice but not barley. Based on the MLSA phylogeny created in the current study, all four strains

isolated by Bowden and Percich (1983) grouped with the Xtu clade, suggesting that the pathovar designation would be *X. translucens* pv. *undulosa* rather than *X. translucens* pv. *cerealis*. The four strains were not tested *in planta* in the current study. The overlapping host range of *X. translucens* pv. *cerealis* and *X. translucens* pv. *undulosa* make it difficult to distinguish these two pathovars. In cases like this, when host range information alone was insufficient, MLSA provided phylogenetic classification that aided in the presumptive identification of *X. translucens* pathovars.

Our findings suggest that cultivated wild rice may also serve as a reservoir for the pathogen that causes BLS in wheat. The four *X. translucens* strains isolated from wild rice in this study grouped in the Xtu clade and exhibited water-soaking on wheat and barley in the *in planta* tests. Wild rice plants were not available, so the pathogenicity of these strains on wild rice was not established in this study. As wild rice is cultivated as a field crop on around 20,000 acres in Minnesota (Office of the Minnesota Secretary of State 2019), with production in the same region as wheat in northern Minnesota, there is potential for mixing of the bacterial populations found on these two hosts where fields of the two crops are in close proximity. However, it should be recognized that this study did not attempt to evaluate any movement of *X. translucens* from one plant species to another, but rather to identify plant hosts growing in proximity to wheat where the bacterium is capable of living.

Twenty-two *Xanthomonas* strains were isolated from wild rice in this study that were not *X. translucens*. These strains appeared not to be pathogenic on wheat or barley in a preliminary *in planta* assay, as they did not exhibit water-soaking on either host (data not shown) and were not further evaluated in the study. Koch's postulates should be

followed to determine if any or all of these strains are pathogens of wild rice. Further studies are needed to identify and characterize these strains. A study including MLSA and *in planta* assays may be useful to elucidate species and/or pathovar designation of these strains.

Relative virulence, along with the character states of strains inoculated on wheat and barley have been used to predict pathovar designations. There is evidence that strains of *X. translucens* pv. *undulosa* are more virulent on wheat, while strains of *X. translucens* pv. *translucens* are more virulent on barley (Adhikari et al. 2012; Curland et al. 2018; Peng et al. 2016). In this study, the relative virulence of strains evaluated in the greenhouse was inconsistent between trials and it did not appear that individual strains were more virulent on wheat, despite the character states supporting a pathovar designation of *X. translucens* pv. *undulosa*. One potential reason for the inconsistent results may be that greenhouse temperatures were lower than is optimal for BLS development, although they were sufficient to determine the pathogenicity of strains. Optimal temperatures for *X. translucens* growth have been reported to be between 27 and 30°C (Bamberg 1936; Duveiller et al. 1997). Also, the trials were conducted at different times of the year (May, October, and November) likely creating inconsistent temperatures and environments among trials. Environmental conditions impact the development of BLS not only in the field but in the greenhouse. This study demonstrated potential challenges in assessing strain virulence on multiple hosts under greenhouse conditions. The development of a greenhouse seedling assay to assess virulence among strains that is reliable and correlates to host reactions in the field would be useful to

identify virulent strains that breeders could use to evaluate germplasm for resistance in a rapid assay.

Future studies on overwintering and epiphytic populations of *X. translucens* on the hosts identified in this study could provide additional information on how the pathogen is surviving from year to year and during the growing season, broadening our understanding of the epidemiology of BLS. Strains that originated on weedy grasses and wild rice appear to be closely related in the Xtu clade based on MLSA, and STs do not appear to be host specific. Crops including wild rice, corn, soybean, and sugarbeet that are grown in the same region as wheat should also be evaluated for their potential to serve as reservoirs for *X. translucens*. In addition, the potential for broadleaf weeds to harbor the pathogen has not been evaluated. A report identifying annual and perennial weed species along with poaceous and non-poaceous plants harboring the fungal pathogen *Fusarium graminearum*, the primary causal agent of Fusarium head blight, demonstrates that the broad host range exhibited by another wheat pathogen influences our understanding of disease epidemiology, ultimately impacting disease control practices (Mourelos et al. 2014).

The LAMP assay designed to detect *X. translucens* pathovars were effective in identifying genetically diverse strains and could be implemented to rapidly survey a wide variety of hosts that may be harboring the pathogen. LAMP assays are robust and can be conducted with minimal equipment, making it more readily useable in field applications (Fischbach et al. 2015; Lang et al. 2014; Langlois et al. 2017). In this study, the *gyrB*-Xt primer set was 100% effective, the Xt-CLS primer set was 95% effective, and the Xt-Cerealis primer set was 100% effective in identifying the STs belonging to the

pathovar groups the primers were designed to detect. LAMP assays could be used to screen early emerging weeds that have been identified as potential reservoirs for the presence of *X. translucens* pathovars that cause BLS on small grains. This information could be useful to target specific species in weed management programs. LAMP assays could be applied in the field to monitor the pathogen population in the wheat crop and surrounding species, aiding in the identification of common virulent strains that breeders might use in selecting wheat varieties with improved resistance. Currently, there are not primers available that can distinguish *X. translucens* pv. *translucens* from *X. translucens* pv. *undulosa*, although these would be useful in a monitoring program. In addition, LAMP assays could be used as a diagnostic tool to detect contaminated seed prior to planting, therefore reducing the amount of inoculum present in the field and the distribution of infested seed locally and internationally.

In conclusion, the findings of this study suggest that weedy grasses and cultivated wild rice may serve as potential reservoirs for *X. translucens* pv. *undulosa*, the pathogen causing BLS on wheat. Identifying weedy species that harbor the pathogen is valuable information for growers, enabling them to establish management strategies that may reduce the amount of inoculum in the field. Monitoring and characterizing the pathogen population of *X. translucens* pv. *undulosa* in Minnesota and the Upper Great Plains is essential for the development of wheat varieties with improved resistance to BLS. The isolation and analysis of *X. translucens* pv. *undulosa* strains from both annual and perennial grass species in Minnesota increases the knowledge of where the pathogen is capable of living and provides insight on the diversity of the pathogen population.

Table 1.1 Summary of the host range of *Xanthomonas translucens* pathovars that cause bacterial leaf streak on small grains.

Xanthomonas species/pathovar	Hosts[a]	Sources
X. translucens pv. *cerealis*	wheat, barley, rye, **smooth brome, quackgrass**	Hagborg 1942; Fang et al. 1950
X. translucens pv. *secalis*	**rye**	Reddy et al. 1924
X. translucens pv. *translucens*	**barley**	Jones et al. 1917
X. translucens pv. *undulosa*	**wheat**, barley, rye, smooth brome, quackgrass	Fang et al. 1950; Smith et al. 1919

[a] Hosts with bold text are considered the natural host(s) of the corresponding *X. translucens* pathovar.

Table 2.2. Location of 18 crop fields in Minnesota where weedy grasses and/or crop plant samples were collected for the isolation of *Xanthomonas spp*.

		Location	
Field ID	Crop[a]	City	County
1	Wheat	Felton	Clay
2	Wheat	New Folden Township	Marshall
3	Wheat	Ada	Norman
4	Wheat	Saint Hilaire	Pennington
5	Wheat	Crookston	Polk
6	Wheat	Crookston	Polk
7	Wheat	Crookston	Polk
8	Wheat	Crookston	Polk
9	Wheat	Crookston	Polk
10	Wheat	Crookston	Polk
11	Wheat	Euclid	Polk
12	Wheat	Fisher	Polk
13	Wheat	Fisher	Polk
14	Wheat, barley[b]	St. Paul	Ramsey
15	Wheat, barley[b]	St. Paul	Ramsey
16	Wheat, barley, oat[b]	St. Paul	Ramsey
17	Wild rice[c]	Washkish Township	Beltrami
18	Wild rice[c]	Washkish Township	Beltrami

[a] Crop(s) growing at that field location.

[b] Experimental fields at the University of Minnesota contained more than one crop identified with natural BLS infection.

[c] Cultivated wild rice fields.

Table 2.3. Host of origin of 87 *Xanthomonas translucens* strains and 47 other *Xanthomonas* species isolated in 2016 and 2017 from weedy grasses and small grain hosts in and around 18 crop fields in Minnesota.

Host of origin	Common name	Number of fields sampled[a]	Number of plants sampled	*Xanthomonas translucens*[b]	*Xanthomonas* spp.[c]
Avena fatua	Wild oat	8	17	11	3
Bromus inermis	Smooth brome	6	8	4	2
Elymus repens	Quackgrass	16	28	37	7
Hordeum jubatum	Foxtail barley	9	22	4	5
Hordeum vulgare	Barley	3	7	6	1
Lolium perenne	Perennial ryegrass	1	4	7	2
Phleum pratense	Timothy	3	4	0	1
Setaria faberi	Giant foxtail	6	12	0	1
Setaria pumila	Yellow foxtail	7	11	0	3
Setaria viridis	Green foxtail	5	11	5	0
Triticum aestivum	Wheat	8	9	9	0
Zizania palustris	Wild rice (cultivated)	7	24	4	22
Total		18	157	87	47

[a] Number of fields sampled out of 18 total fields.

[b] Number of strains predicted to be *X. translucens* by 16S rDNA sequence.

[c] Number of strains predicted to be *Xanthomonas* species other than *translucens* by 16S rDNA sequence.

Table 2.4. List of described *Xanthomonas translucens* strains used as reference strains for comparison of strains isolated in this study.

Strain ID[a]	Species and pathovar designation	Host of origin	Geographic origin	Source[b]
CIX40	*X. translucens* pv. *undulosa*	*Triticum aestivum*	Minnesota	Curland et al. 2018
CIX41	*X. translucens* pv. *translucens*	*Hordeum vulgare*	Minnesota	Curland et al. 2018
CIX43	*X. translucens* pv. *translucens*	*Hordeum vulgare*	Minnesota	Curland et al. 2018
CIX84	*X. translucens* pv. *translucens*	*Hordeum vulgare*	Minnesota	Curland et al. 2018
CIX95	*X. translucens* pv. *translucens*	*Hordeum vulgare*	Minnesota	Curland et al. 2018
LMG 679^{PT}	*X. translucens* pv. *cerealis*	*Bromus inermis*	United States	BCCM/LMG
LMG 726^{PT}	*X. translucens* pv. *graminis*	*Dactylis glomerata*	Switzerland	BCCM/LMG
LMG 876^{T}	*X. translucens* pv. *translucens*	*Hordeum vulgare*	Minnesota	BCCM/LMG
LMG 883^{PT}	*X. translucens* pv. *undulosa*	*Triticum turgidum*	Canada	BCCM/LMG
LMG 892^{PT}	*X. translucens* pv. *secalis*	*Secale cereale*	Canada	BCCM/LMG
Xt-2	*X. campestris* pv. *cerealis*[c]	*Elymus repens*	Minnesota	Bowden & Percich 1983
Xt-8	*X. campestris* pv. *cerealis*[c]	*Zizania palustris*	Minnesota	Bowden & Percich 1983
Xt-22	*X. campestris* pv. *cerealis*[c]	*Zizania palustris*	Minnesota	Bowden & Percich 1983
Xt-25	*X. campestris* pv. *cerealis*[c]	*Elymus repens*	Minnesota	Bowden & Percich 1983

[a] Type (^{T}) and pathotype (^{PT}) strains.

[b] BCCM/LMG = Belgian Co-ordinated Collections of Micro-organisms.

[c] Species and pathovar designations are listed as described by Bowden & Percich (1983).

Table 2.5. Multilocus sequence analysis (MLSA) clade and sequence type of 87 *X. translucens* strains originating from various hosts isolated in 2016 and 2017 and 14 reference strains including type and pathotype strains of *X. translucens.*

Strain ID[a]	Host of origin	Year of isolation	Field ID[b]	MLSA clade[c]	Sequence type[d]
CIX207	*Avena fatua*	2017	10	Xtu	34
CIX188-191, CIX196	*Avena fatua*	2017	8	Xtu	36
CIX228	*Avena fatua*	2017	12	Xtu	36
CIX288	*Avena fatua*	2016	6	Xtu	36
CIX289-290	*Avena fatua*	2016	7	Xtu	36
CIX297	*Avena fatua*	2016	4	Xtu	38
LMG 679^{PT}	*Bromus inermis*	1941	-	Xtc	60
CIX180	*Bromus inermis*	2017	3	Xtc	60
CIX177-178	*Bromus inermis*	2017	3	Xtc	61
CIX179	*Bromus inermis*	2017	3	Xtc	62
LMG 726^{PT}	*Dactylis glomerata*	1973	-	Xtg	63
CIX175-176	*Elymus repens*	2017	3	Xtu	30
CIX301	*Elymus repens*	2016	2	Xtu	30
Xt-2, Xt-25	*Elymus repens*	1981	-	Xtu	36
CIX169, CIX173-174	*Elymus repens*	2017	3	Xtu	36
CIX184-187, CIX194-195	*Elymus repens*	2017	8	Xtu	36
CIX220-227	*Elymus repens*	2017	12	Xtu	36
CIX333-335	*Elymus repens*	2016	18	Xtu	36
CIX279-281	*Elymus repens*	2016	5	Xtu	38
CIX192-193	*Elymus repens*	2017	8	Xtu	53
CIX210-215	*Elymus repens*	2017	10	Xtu	53
CIX323-325	*Elymus repens*	2016	17	Xtu	54
CIX282-283	*Hordeum jubatum*	2016	5	Xtu	29
CIX232-233	*Hordeum jubatum*	2017	13	Xtu	36
LMG 876^{T}	*Hordeum vulgare*	1933	-	Xtt A	15
CIX261	*Hordeum vulgare*	2016	14	Xtt A	15
CIX43	*Hordeum vulgare*	2009	-	Xtt A	24
CIX266-267	*Hordeum vulgare*	2016	14	Xtt A	24
CIX84	*Hordeum vulgare*	2011	-	Xtt B	43
CIX258	*Hordeum vulgare*	2017	16	Xtt B	43
CIX259	*Hordeum vulgare*	2017	15	Xtt B	43
CIX41	*Hordeum vulgare*	2009	-	Xtt C	23
CIX95	*Hordeum vulgare*	2011	-	Xtt C	23
CIX260	*Hordeum vulgare*	2016	14	Xtt C	23

Table 2.4. *Continued from previous page.*

CIX236-237, CIX239	*Lolium perenne*	2017	13	Xtu	30
CIX238, CIX240-242	*Lolium perenne*	2017	13	Xtu	56
LMG 883^{PT}	*Secale cereale*	1966	-	Xtu	33
CIX161	*Setaria viridis*	2017	1	Xtu	36
CIX171	*Setaria viridis*	2017	1	Xtu	54
CIX162-163, CIX170	*Setaria viridis*	2017	1	Xtu	56
CIX40	*Triticum aestivum*	2010	-	Xtu	36
CIX181	*Triticum aestivum*	2017	8	Xtu	36
CIX197	*Triticum aestivum*	2017	9	Xtu	36
CIX216	*Triticum aestivum*	2017	12	Xtu	36
CIX229	*Triticum aestivum*	2017	13	Xtu	36
CIX272	*Triticum aestivum*	2016	14	Xtu	36
CIX271	*Triticum aestivum*	2016	14	Xtu	38
CIX154	*Triticum aestivum*	2017	1	Xtu	53
CIX164	*Triticum aestivum*	2017	1	Xtu	57
CIX198	*Triticum aestivum*	2017	11	Xtu	59
LMG 892^{PT}	*Triticum turgidum*	1966	-	Xtu	36
CIX303-304	*Zizania palustris*	2016	17	Xtu	30
Xt-22	*Zizania palustris*	1981	-	Xtu	36
CIX306, CIX308	*Zizania palustris*	2016	17	Xtu	54
Xt-8	*Zizania palustris*	1981	-	Xtu	58

[a] Type (^{T}) and pathotype (^{PT}) strains.

[b] Field identification number listed are as described in Table 2.1 and are only provided for the strains isolated in this study.

[c] MLSA clade was based on previously described phylogenies of the concatenated sequence of four loci (*rpoD, dnaK*, *fyuA*, and *gyrB*) as described by Curland et al. (2018).

[d] Sequence types were assigned according to each unique concatenated sequence of the four loci used in this study (*rpoD*, *dnaK*, *fyuA*, and *gyrB*).

Table 2.6. Back-transformed mean lesion lengths (cm) on wheat (cv. RB07) and barley (cv. Quest) seedling leaves infiltrated with *X. translucens* strains collected in 2016 and 2017 in greenhouse trials. Lesion length was assessed 9-10 days post inoculation.

	Mean lesion length (cm)[z]			
	2016 collection		2017 collection	
Host crop	Trial 1	Trial 2	Trial 1	Trial 2
Wheat	3.57[a]	1.84[b]	2.24[b]	1.20[b]
Barley	2.57[b]	2.68[a]	2.98[a]	2.09[a]

[z] Means within a column followed by a different letter were significantly different based on Tukey's HSD at $P < 0.05$.

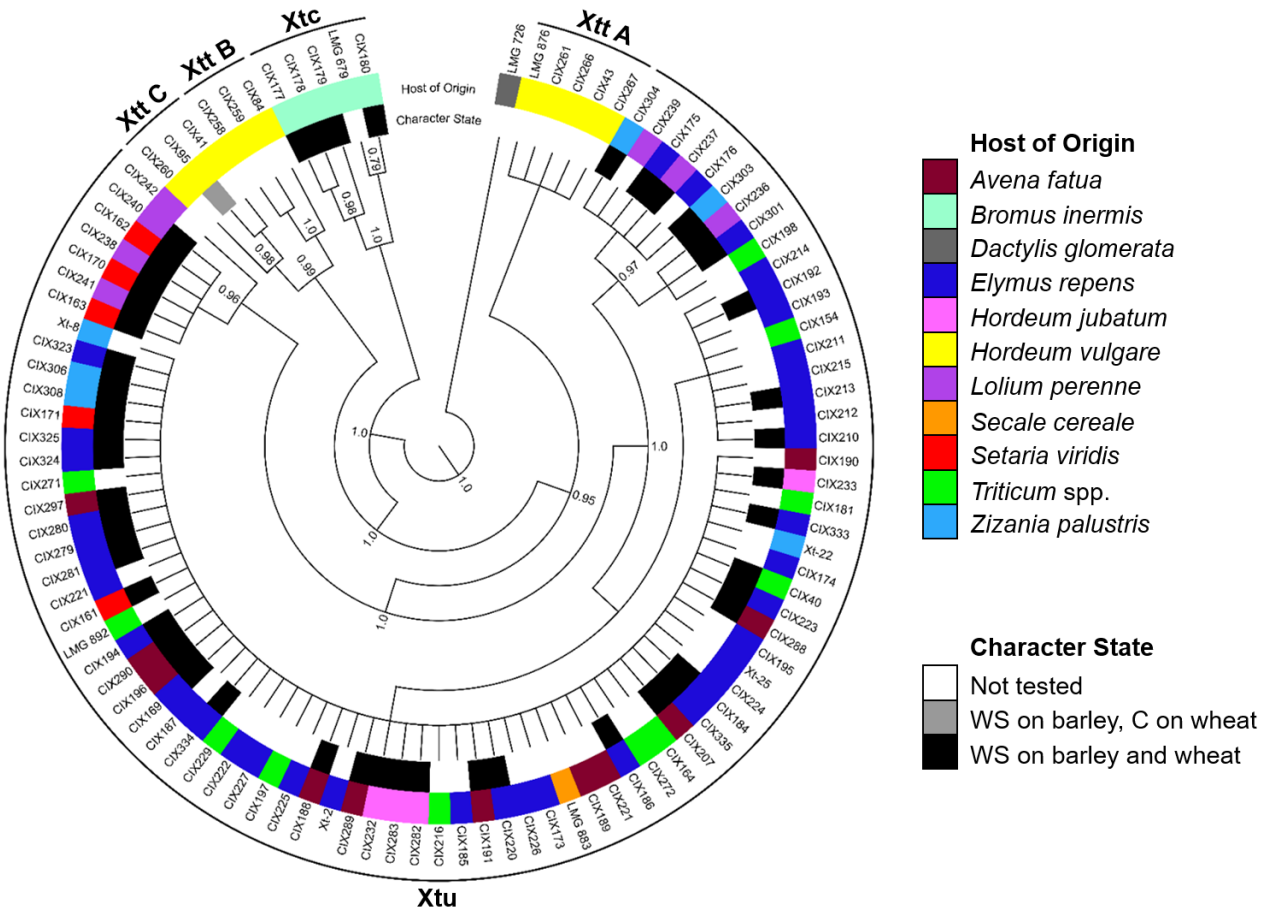


Figure 2.1. Circular cladogram of Bayesian analysis of concatenated sequences from *rpoD*, *dnaK*, *fyuA*, and *gyrB* (2,645 bp) housekeeping genes for 101 *Xanthomonas translucens* strains, including reference strains LMG 679^{PT} *X. translucens* pv. *cerealis*, LMG 726^{PT} *X. translucens* pv. *graminis*, LMG 883^{PT} *X. translucens* pv. *secalis*, LMG 876^{T} *X. translucens* pv. *translucens*, and LMG 892^{PT} *X. translucens* pv. *undulosa*. The cladogram shows five distinct clades, *X. translucens* pv. *cerealis* (Xtc), *X. translucens* pv. *translucens* clades A (Xtt A), B (Xtt B), and C (Xtt C), and *X. translucens* pv. *undulosa* (Xtu). Posterior probabilities > 0.70 are labeled at nodes. Host of origin and character states (WS = water-soaking and C = chlorosis) are included as metadata.

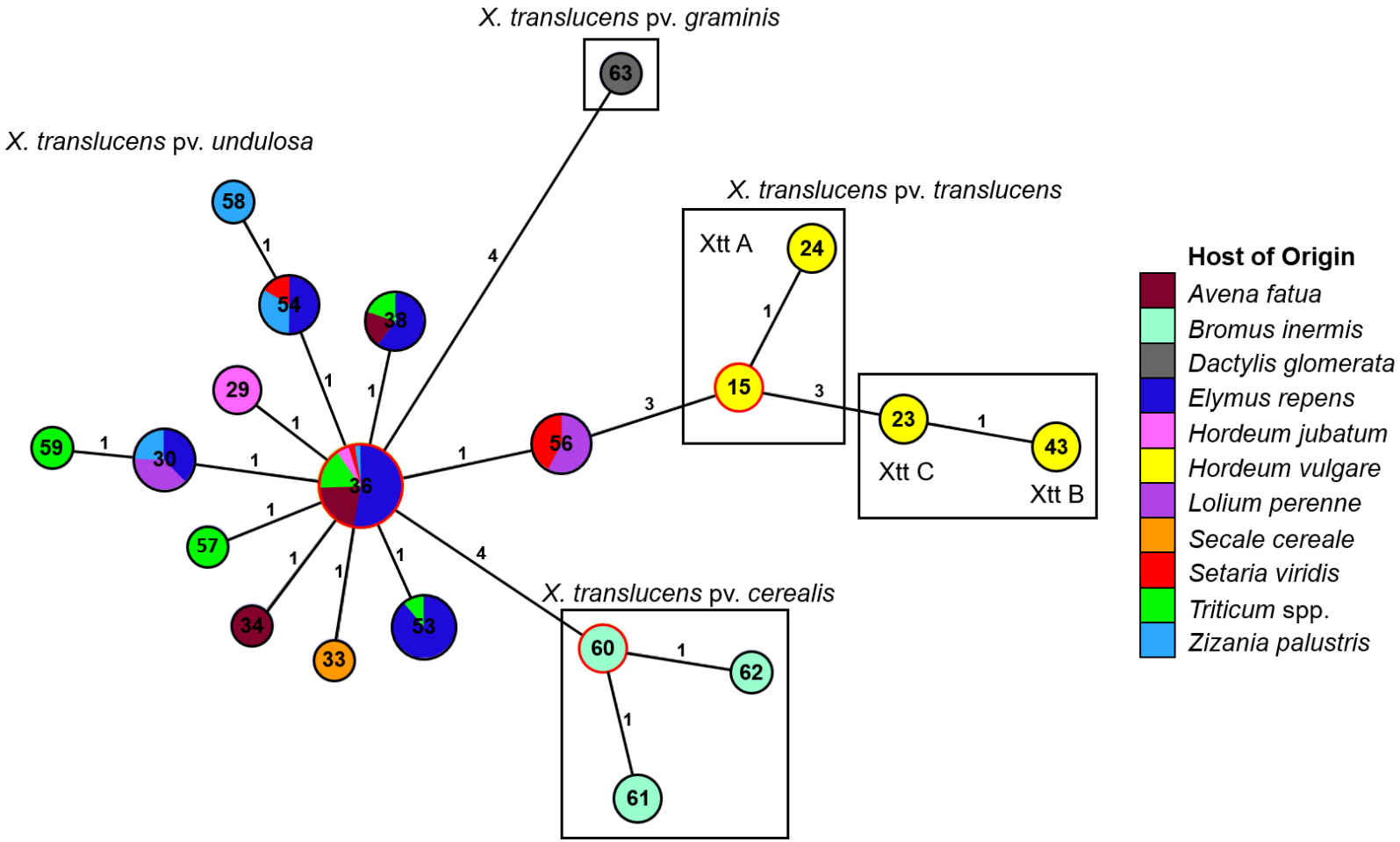


Figure 2.2. Minimal spanning tree created in PHYLOViZ 2.0 of 101 *Xanthomonas translucens* strains originating from various hosts depicting five clonal complexes. Numbers within circles indicate sequence type (ST) based on the unique concatenated sequence of four genes (*rpoD*, *dnaK*, *fyuA*, and *gyrB*). Numbers on branches indicate the number of allelic variances (out of four) with 1 equal to a single locus variant and 4 equal to no identical alleles. Circles outlined in red represent the founding ST for the pathovar group.

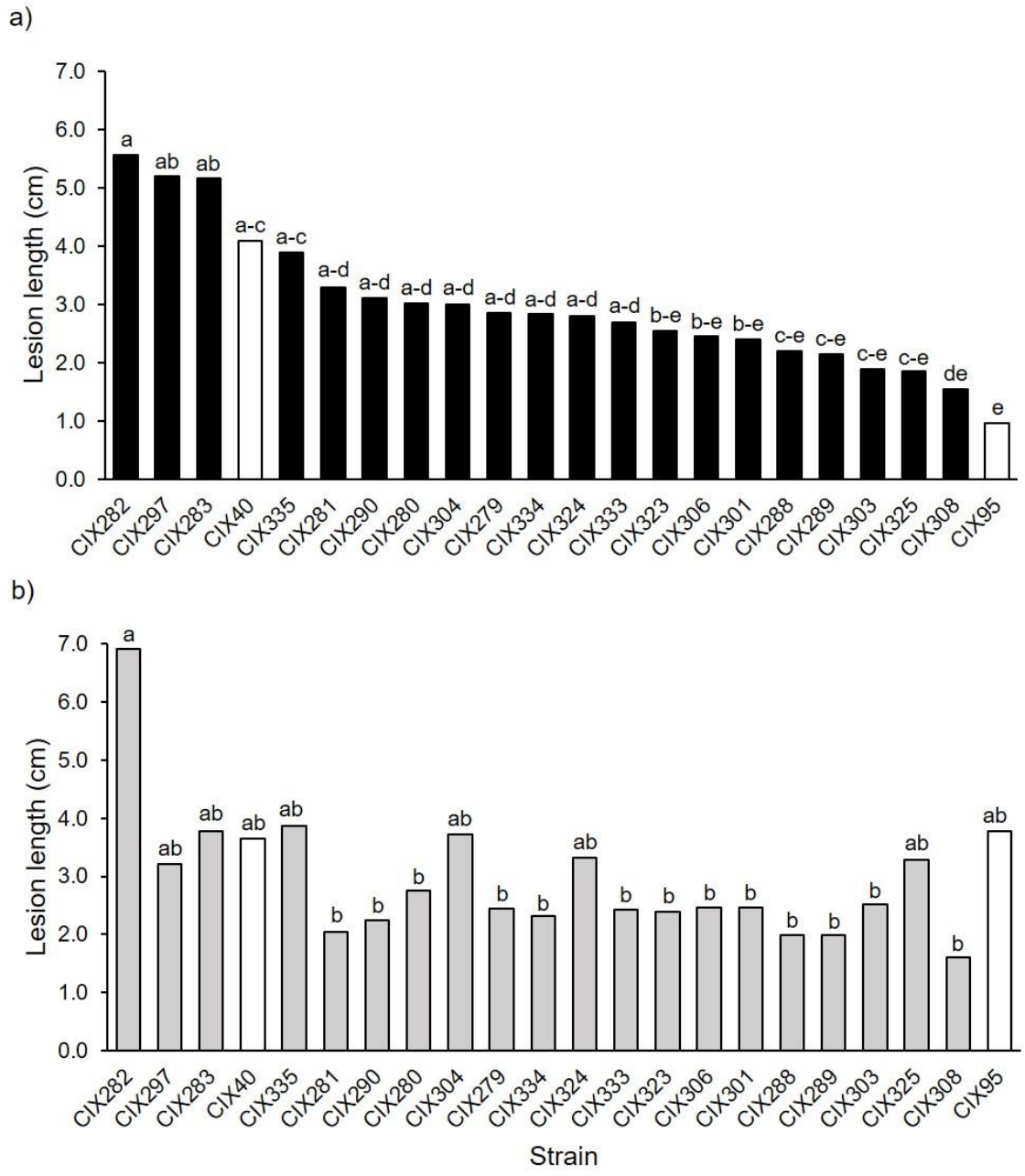


Figure 2.3. Back-transformed mean lesion lengths of 20 strains from various grasses and cultivated wild rice isolated in 2016 and two described strains, CIX40 (*X. translucens* pv. *undulosa*) and CIX95 (*X. translucens* pv. *translucens*) (Curland et al. 2018), infiltrated into **a)** wheat (cv. RB07) and **b)** barley (cv. Quest) seedling leaves. Lesion length was assessed 9-10 days post inoculation. Previously described strains are indicated by white bars. Letters above bars represent mean separation based on Tukey's HSD test ($P < 0.05$).

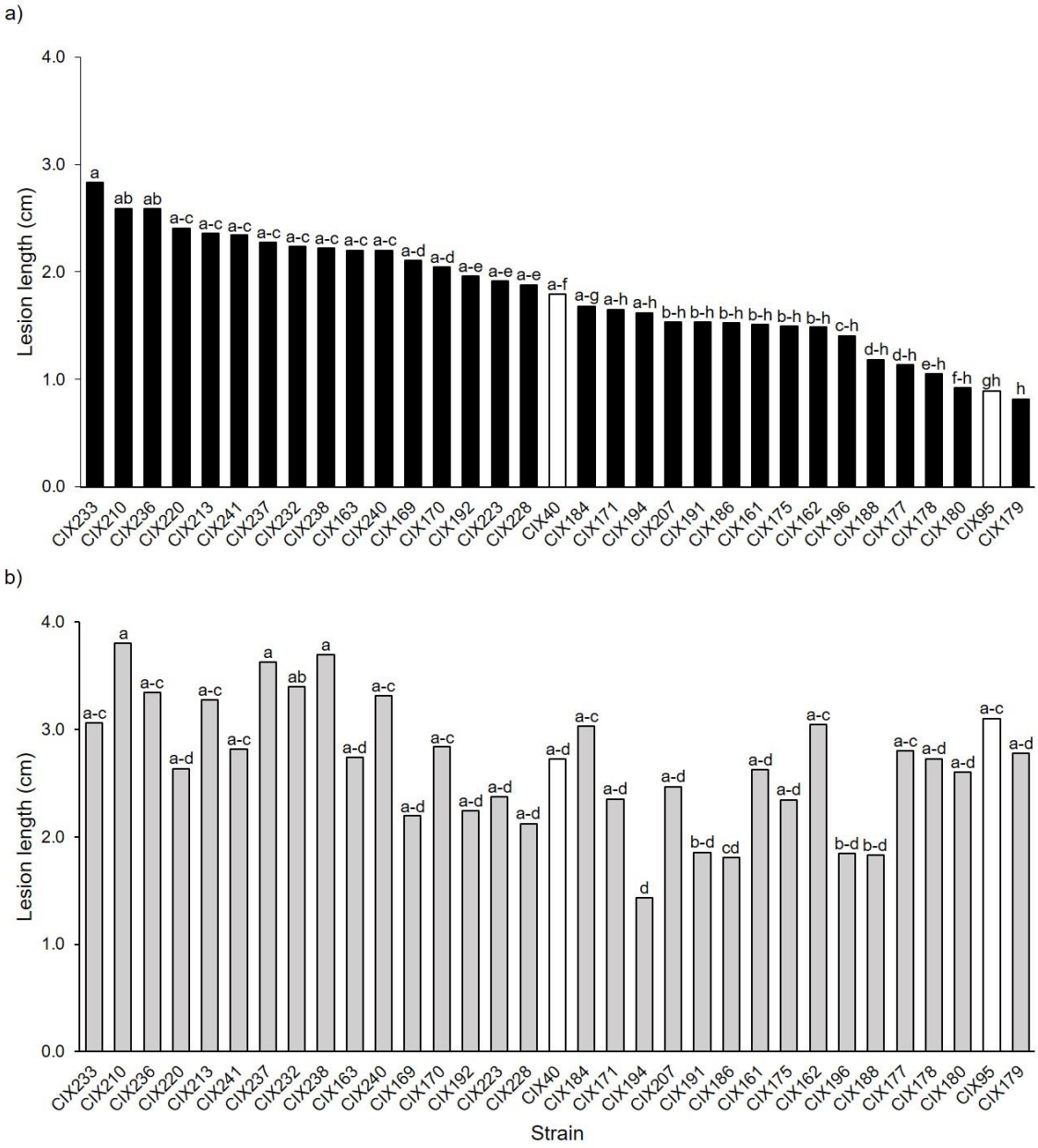


Figure 2.4. Back-transformed mean lesion lengths of 31 strains from various grasses isolated in 2017 and two described strains, CIX40 (*X. translucens* pv. *undulosa*) and CIX95 (*X. translucens* pv. *translucens*) (Curland et al. 2018), infiltrated in **a)** wheat (cv. RB07) and **b)** barley (cv. Quest) seedling leaves. Lesion length was assessed 9-10 days post inoculation. Previously described strains are indicated by white bars. Letters above bars represent mean separation based on Tukey's HSD test ($P < 0.05$).

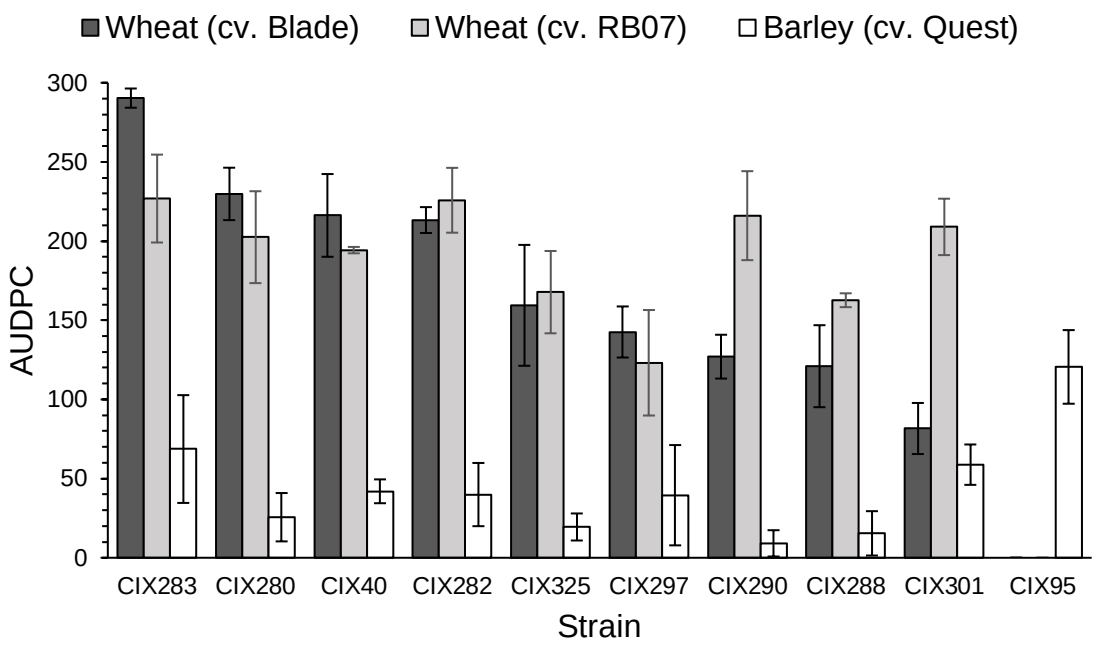


Figure 2.5. AUDPC values of eight unknown strains isolated from weedy grasses and two known *X. translucens* strains, CIX40 (*X. translucens* pv. *undulosa*) and CIX95 (*X. translucens* pv. *translucens*), spray inoculated onto wheat cv. Blade, wheat cv. RB07 and barley cv. Quest. The field testing was conducted in 2017 and plots were assessed at 10, 13, and 17 days post inoculation. Error bars represent standard error across the four replications of each treatment.

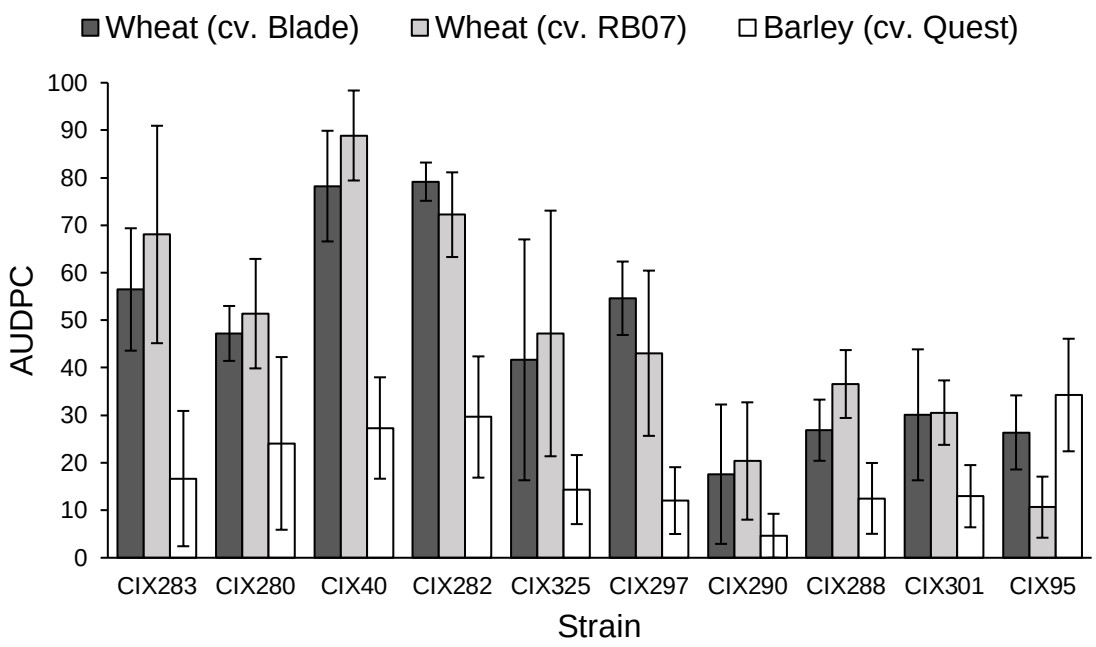


Figure 2.6. AUDPC values of eight unknown strains isolated from weedy grasses and two known *X. translucens* strains, CIX40 (*X. translucens* pv.*undulosa*) and CIX95 (*X. translucens* pv. *translucens*), spray inoculated onto wheat cv. Blade, wheat cv. RB07 and barley cv. Quest. The field testing was conducted in 2018 and plots were assessed at 7 and 10 days post inoculation. Error bars represent standard error across the four replications of each treatment.

References

Adhikari, T. B., Gurung, S., Hansen, J. M., & Bonman, J. M. (2012). Pathogenic and genetic diversity of *Xanthomonas translucens* pv. *undulosa* in North Dakota. *Phytopathology*, *102*(4), 390–402.

Adhikari, T. B., Hansen, J. M., Gurung, S., & Bonman, J. M. (2011). Identification of new sources of resistance in winter wheat to multiple strains of *Xanthomonas translucens* pv. *undulosa*. *Plant Disease*, *95*(5), 582–588.

Almeida, N. F., Yan, S., Cai, R., Clarke, C. R., Morris, C. E., Schaad, N. W., Schuenzel, E. L., Lacy, G. H., Sun, X., Jones, J. B., Castillo, J. A., Bull, C. T., Leman, S., Guttman, D. S., Setubal, J. C., & Vinatzer, B. A. (2010). PAMDB, a multilocus sequence typing and analysis database and website for plant-associated microbes. *Phytopathology*, *100*(42), 208–215.

Bamberg, R. H. (1936). Black chaff disease of wheat. *Journal of Agricultural Research*, *52*(6), 397–417.

Boosalis, M. G. (1952). The epidemiology of *Xanthomonas translucens* (J. J. and R.) Dowson on cereals and grasses. *Phytopathology*, *42*, 387–395.

Bowden, R. L., & Percich, J. A. (1983). Etiology of bacterial leaf streak of wild rice. *Phytopathology*, *73*(5), 640–645.

Bragard, C., Singer, E., Alizadeh, A., Vauterin, L., Maraite, H., & Swings, J. (1997). *Xanthomonas translucens* from small grains: Diversity and phytopathological relevance. *Phytopathology*, *87*(11), 1111–1117.

Braun, H. (1920). Presoak method of seed treatment: A means of preventing seed injury due to chemical disinfectants and of increasing germicidal efficiency. *Journal of Agricultural Research*, *19*(8), 363–392.

Bull, C. T., Clarke, C. R., Cai, R., Vinatzer, B. A., Jardini, T. M., & Koike, S. T. (2011). Multilocus sequence typing of *Pseudomonas syringae* sensu lato confirms previously described genomospecies and permits rapid identification of *P. syringae* pv. *coriandricola* and *P. syringae* pv. *apii* causing bacterial leaf spot on parsley. *Phytopathology*, *101*(7), 847–858.

Bull, C. T., De Boer, S. H., Denny, T. P., Firrao, G., Fischer-Le Saux, M., Saddler, G. S., Scortichini, M., Stead, D. E., & Takikawa, Y. (2010). Comprehensive list of names of plant pathogenic bacteria, 1980-2007. *Journal of Plant Pathology*, *92*(3), 551–592.

Croce, V., Pianzzola, M. J., Durand, K., González-Arcos, M., Jacques, M. A., & Siri, M. I. (2016). Multilocus sequence typing reveals high variability among *Clavibacter michiganensis* subsp. *michiganensis* strains affecting tomato crops in Uruguay. *European Journal of Plant Pathology*, *144*, 1–13.

Curland, R. D., Gao, L., Bull, C. T., Vinatzer, B. A., Dill-macky, R., Van Eck, L., & Ishimaru, C. A. (2018). Genetic diversity and virulence of wheat and barley strains of *Xanthomonas translucens* from the upper Midwestern United States. *Phytopathology*, *108*(4), 443–453.

de Mendiburu, F. (2019). *agricolae: Statistical procedures for agricultural research. R package version 1.3-0*.

Dowson, W. J. (1939). On the systematic position and generic names of the gram negative bacterial plant pathogens. *Zentralblatt Fuer Bakt. Etc II. Abt.*, *100*(9/13), 177–193.

Drummond, A. J., Suchard, M. A., Xie, D., & Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, *29*, 1969–1973.

Duveiller, E. (1994a). A pictorial series of disease assessment keys for bacterial leaf streak of cereals. *Plant Disease*, *78*(2), 137–141.

Duveiller, E. (1994b). Bacterial leaf streak or black chaff of cereals. *Bulletin OEPP/EPPO*, *24*, 135–157.

Duveiller, E., Fucikovsky, L., & Rudolph, K. (Eds.). (1997). *The Bacterial Diseases of Wheat: Concepts and Methods of Disease Management*. Mexico, D.F.: CIMMYT.

Duveiller, E., Kandel, Y. R., Sharma, R. C., & Shrestha, S. M. (2005). Epidemiology of foliar blights (spot blotch and tan spot) of wheat in the plains bordering the Himalayas. *Phytopathology*, *95*(3), 248–256.

Dye, D. W. (1962). The inadequacy of the usual determinative tests for the identification of *Xanthomonas* spp. *New Zealand Journal of Science*, *5*(4), 393–416.

Dye, D. W., Bradbury, J. F., Goto, M., Hayward, A. C., Lelliott, R. A., & Schroth, M. N. (1980). International standards for naming pathovars of phytopathogenic bacteria and a list of pathovar names and pathotype strains. *Review of Plant Pathology*, *59*(4), 153–159.

Dye, D. W., & Lelliott, R. A. (1974). Genus II. *Xanthomonas* Dowson 1939. In R. E.

Buchanan & N. E. Gibbons (Eds.), *Bergey's Manual of Determinative Bacteriology* (8th ed., pp. 243–249). Baltimore: Williams & Wilkins.

Egli, T., Goto, M., & Schmidt, D. (1975). Bacterial wilt, a new forage grass disease. *Phytopathologische Zeitschrift, 82*, 111–121.

Egli, T., & Schmidt, D. (1982). Pathogenic variation among the causal agents of bacterial wilt of forage grasses. *Phytopathologische Zeitschrift, 104*, 138–150.

Fang, C. T., Allen, O. N., Riker, A. J., & Dickson, J. G. (1950). The pathogenic, physiological, and serological reactions of the form species of *Xanthomonas translucens*. *Phytopathology, 40*, 44–64.

Feil, E. J., Li, B. C., Aanensen, D. M., Hanage, W. P., & Spratt, B. G. (2004). eBURST : Inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. *Journal of Bacteriology, 186*(5), 1518–1530.

Fischbach, J., Xander, N. C., Frohme, M., & Glokler, J. F. (2015). Shining a light on LAMP assays - A comparison of LAMP visualization methods including the novel use of berberine. *BioTechniques, 58*(4), 189–194.

Forster, R. L. (1982). The status of black chaff disease in Idaho. *Idaho Wheat, 12*, 3–4.

Gevers, D., Cohan, F. M., Lawrence, J. G., Spratt, B. G., Coenye, T., Feil, E. J., … Swings, J. (2005). Re-evaluating prokaryotic species. *Nature Reviews Microbiology, 3*, 733–739.

Hagborg, W. A. F. (1942). Classification revision in *Xanthomonas translucens*. *Canadian Journal of Research, 20*(Sec. C), 312–326.

Hauben, L., Vauterin, L., Swings, J., & Moore, E. R. B. (1997). Comparison of 16S ribosomal DNA sequences of all *Xanthomonas* species. *International Journal of Systematic Bacteriology*, *47*(2), 328–335.

Hayward, A. C. (1993). The hosts of *Xanthomonas*. In J. G. Swings & E. L. Civerolo (Eds.), *Xanthomonas* (1st ed., pp. 1–17). Chapman & Hall.

Jeanes, A., Pittsley, J. E., & Senti, F. R. (1961). Polysaccharide B-1459: A new hydrocolloid polyelectrolyte produced from glucose by bacterial fermentation. *Journal of Applied Polymer Science*, *5*(17), 519–526.

Jones, L. R., Johnson, A. G., & Reddy, C. S. (1916). Bacterial blights of barley and certain other cereals. *Science*, *44*(1134), 432–433.

Jones, L. R., Johnson, A. G., & Reddy, C. S. (1917). Bacterial-blight of barley. *Journal of Agricultural Research*, *11*(12), 625–651.

Kandel, Y. R., Glover, K. D., Tande, C. A., & Osborne, L. E. (2012). Evaluation of spring wheat germplasm for resistance to bacterial leaf streak caused by *Xanthomonas campestris* pv. *translucens*. *Plant Disease*, *96*(12), 1743–1748.

Kernkamp, M. F., Kroll, R., & Woodruff, W. C. (1976). Diseases of cultivated wild rice in Minnesota. *Plant Disease Reporter*, *60*(9), 771–775.

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest package: Tests in linear mixed effects models. *Journal of Statistical Software*, *82*(13), 1–26.

Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics* (pp. 125–175). Chichester: Wiley.

Lang, J. M., Langlois, P., Nguyen, M. H. R., Triplett, L. R., Purdie, L., Holton, T. A.,

Djikeng, A., Vera Cruz, C. M., Verdier, V., & Leach, J. E. (2014). Sensitive detection of *Xanthomonas oryzae* pathovars *oryzae* and *oryzicola* by loop-mediated isothermal amplification. *Applied and Environmental Microbiology*, *80*(15), 4519–4530.

Langlois, P. A., Snelling, J., Hamilton, J. P., Bragard, C., Koebnik, R., Triplett, L. R., Blom, J., Tisserat, N. A., & Leach, J. E. (2017). Characterization of the *Xanthomonas translucens* complex using draft genomes, comparative genomics, phylogenetic analysis, and diagnostic LAMP assays. *Phytopathology*, *107*, 519–527.

Maiden, M. C., Bygraves, J. A., Feil, E., Morelli, G., Russell, J. E., Urwin, R., Zhang, Q., Zhou, J., Zurth, K., Caugant, D. A., Feavers, I. M., Achtman, M., & Spratt, B. G. (1998). Multilocus sequence typing: A portable approach to the identification of clones within populations of pathogenic microorganisms. *Proceedings of the National Academy of Sciences of the United States of America*, *95*, 3140–3145.

Minnesota Agricultural Experiment Station. (2012). *2011 Minnesota field crop trials.* St. Paul, MN: Agricultural Experiment Station, University of Minnesota.

Minnesota Agricultural Experiment Station. (2017). *2017 Minnesota field crop trials*. St. Paul, MN: Minnesota Agricultural Experiment Station, University of Minnesota.

Mourelos, C. A., Malbran, I., Balatti, P. A., Ghiringhelli, P. D., & Lori, G. A. (2014). Gramineous and non-gramineous weed species as alternative hosts of *Fusarium graminearum*, causal agent of *Fusarium* head blight of wheat, in Argentina. *Crop Protection*, *65*, 100–104.

Nascimento, M., Sousa, A., Ramirez, M., Francisco, A. P., Carrico, J. A., & Vaz, C.

(2017). PHYLOViZ 2.0 : providing scalable data integration and visualization for multiple phylogenetic inference methods. *Bioinformatics*, *33*(1), 128–129.

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N., & Hase, T. (2000). Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research*, *28*(12).

Office of the Minnesota Secretary of State. (2019). State grain - Wild rice. Retrieved from https://www.sos.state.mn.us/about-minnesota/state-symbols/state-grain-wild-rice/

Peng, Z., Hu, Y., Xie, J., Potnis, N., Akhunova, A., Jones, J., Liu, Z., White, F. F., & Liu, S. (2016). Long read and single molecule DNA sequencing simplifies genome assembly and TAL effector gene analysis of *Xanthomonas translucens*. *BMC Genomics*, *17*(21), 1–19.

Rambaut, A., Suchard, M. A., Xie, D., & Drummond, A. J. (2014). *Tracer v1.6*.

Reddy, C. S., Godkin, J., & Johnson, A. G. (1924). Bacterial blight of rye. *Journal of Agricultural Research*, *28*(10), 1039–1040.

Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J. C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S. E., & Sánchez-Gracia, A. (2017). DnaSP 6: DNA sequence polymorphism analysis of large datasets. *Molecular Biology and Evolution, 34,* 3299–3302.

Saari, E. E., & Prescott, J. M. (1975). A scale for appraising the foliar intensity of wheat diseases. *Plant Disease Reporter*, *59*(5), 377–380.

Schaad, N. W., & Stall, R. E. (1988). *Xanthomonas*. In N. W. Schaad (Ed.), *Laboratory Guide for Identification of Plant Pathogenic Bacteria* (2nd ed., pp. 81–94). St. Paul:

APS Press.

Smith, E. F., Jones, L. R., & Reddy, C. S. (1919). The black chaff of wheat. *Science*, *50*(1280), 48.

Starr, M. P., Jenkins, C. L., Bussey, L. B., & Andrewes, A. G. (1977). Chemotaxonomic significance of the xanthomonadins, novel brominated aryl-polyene pigments produced by bacteria of the genus *Xanthomonas*. *Archives of Microbiology*, *113*, 1–9.

Thompson, D. C., Schaad, N. W., & Forster, R. L. (1989). New perennial hosts of epiphytic populations of *Xanthomonas campestris* pv. *translucens*. (Abstr.). *Phytopathology*, *79*, S1168.

Tubajika, K. M., Tillman, B. L., Russin, J. S., Clark, C. A., & Harrison, S. A. (1998). Relationship between flag leaf symptoms caused by *Xanthomonas translucens* pv. *translucens* and subsequent seed transmission in wheat. *Plant Disease*, *82*(12), 1341–1344.

USDA. (2017). Quick Stats Lite. Retrieved from http://usda.gov/Quick_Stats/Lite/index.php

Vauterin, L., Hoste, B., Kersters, K., & Swings, J. (1995). Reclassification of *Xanthomonas*. *International Journal of Systematic Bacteriology*, *45*(3), 472–489.

Vauterin, L., Yang, P., Hoste, B., Pot, B., Swings, J., & Kersters, K. (1992). Taxonomy of xanthomonads from cereals and grasses based on SDS- PAGE of proteins, fatty acid analysis and DNA hybridization. *Journal of General Microbiology*, *138*, 1467–1477.

Villari, C., Tomlinson, J. A., Battisti, A., Boonham, N., Capretti, P., & Faccoli, M. (2013). Use of loop-mediated isothermal amplification for detection of *Ophiostoma clavatum*, the primary blue stain fungus associated with *Ips acuminatus*. *Applied and Environmental Microbiology*, *79*(8), 2527–2533.

Waldron, L. R. (1929). The relationship of black chaff disease of wheat to certain physical and pathological characters. *Science*, *70*(1811), 268.

Wallin, J. R. (1946). Parasitism of *Xanthomonas translucens* (J. J. and R.) Dowson on grasses and cereals. *Iowa State College Journal of Science*, *20*, 171–193.

Wallin, J. R., & Reddy, C. S. (1945). A bacterial streak disease of *Phleum pratense* L. *Phytopathology*, *35*, 937–939.

Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E., Stackebrandt, E. & Truper, H. G. (1987). Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *International Journal of Systematic Bacteriology*, *37*(4), 463–

Young, J. M., Dye, D. W., Bradbury, J. F., Panagopoulos, C. G., & Robbs, C. F. (1978). A proposed nomenclature and classification for plant pathogenic bacteria. *New Zealand Journal of Agricultural Research*, *21*, 153–177.

Young, J. M., Park, D. C., Shearman, H. M., & Fargier, E. (2008). A multilocus sequence analysis of the genus *Xanthomonas*. *Systematic and Applied Microbiology*, *31*(5),

Zacaroni, A. B., Koike, S. T., De souza, R. M., & Bull, C. T. (2012). Bacterial leaf spot of radicchio (*Cichorium intybus*) is caused by *whe*. *Plant Disease*, *96*, 1820.

Appendices

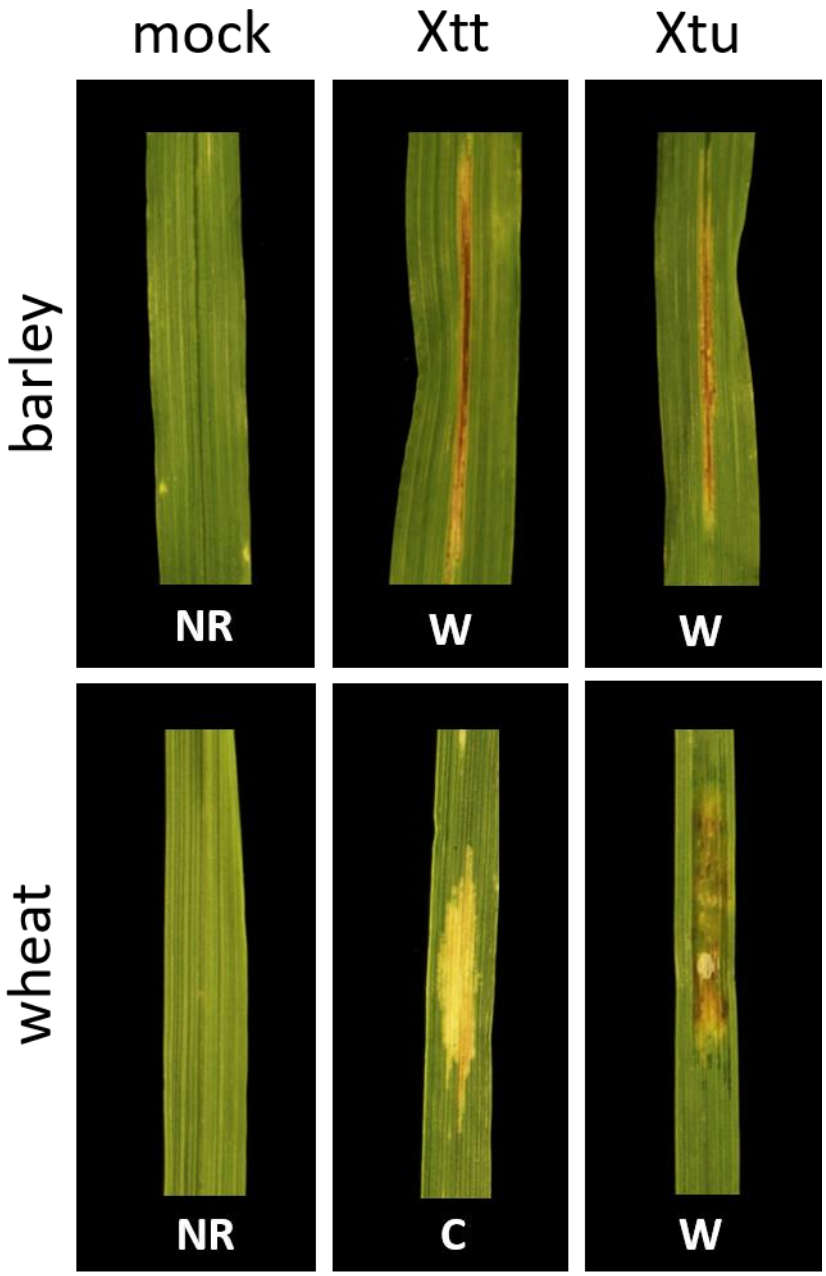
Appendix Table 2.1. List of 33 *Xanthomonas* strains representing 24 different sequence types used in loop-mediated isothermal amplification assays.

Xanthomonas species/pathovar	Strain ID[a]	ST[b]	Source[c]
X. translucens pv. *undulosa*	CIX282	29	This study
X. translucens pv. *undulosa*	CIX301	30	This study
X. translucens pv. *undulosa*	CIX207	34	This study
X. translucens pv. *undulosa*	LMG 892^{PT}	36	BCCM/LMG
X. translucens pv. *undulosa*	CIX288	36	This study
X. translucens pv. *undulosa*	CIX192	53	This study
X. translucens pv. *undulosa*	CIX323	54	This study
X. translucens pv. *undulosa*	CIX238	56	This study
X. translucens pv. *undulosa*	CIX164	57	This study
X. translucens pv. *undulosa*	CIX198	59	This study
X. translucens pv. *undulosa*	CIX297	38	This study
X. translucens pv. *translucens* (Xtt A)	LMG 876^{T}	15	BCCM/LMG
X. translucens pv. *translucens* (Xtt A)	CIX261	15	This study
X. translucens pv. *translucens* (Xtt A)	CIX43	24	Curland et al. 2018
X. translucens pv. *translucens* (Xtt A)	CIX266	24	This study
X. translucens pv. *translucens* (Xtt A)	CIX267	24	This study
X. translucens pv. *translucens* (Xtt A)	CIX34	25	Curland et al. 2018
X. translucens pv. *translucens* (Xtt A)	CIX76	31	Curland et al. 2018
X. translucens pv. *translucens* (Xtt B)	CIX132	41	Curland et al. 2018
X. translucens pv. *translucens* (Xtt B)	CIX26	42	Curland et al. 2018
X. translucens pv. *translucens* (Xtt B)	CIX20	43	Curland et al. 2018
X. translucens pv. *translucens* (Xtt B)	CIX258	43	This study
X. translucens pv. *translucens* (Xtt C)	CIX126	16	Curland et al. 2018
X. translucens pv. *translucens* (Xtt C)	CIX260	23	This study
X. translucens pv. *cerealis*	LMG 679^{PT}	60	BCCM/LMG
X. translucens pv. *cerealis*	CIX180	60	This study
X. translucens pv. *cerealis*	CIX177	61	This study
X. translucens pv. *cerealis*	CIX178	61	This study
X. translucens pv. *cerealis*	CIX179	62	This study
X. translucens pv. *secalis*	LMG 883^{PT}	33	BCCM/LMG
X. translucens pv. *graminis*	LMG 726^{PT}	63	BCCM/LMG
X. translucens pv. *poae*	LMG 728^{PT}	-	BCCM/LMG
Xanthomonas sp.	CIX286	-	This study

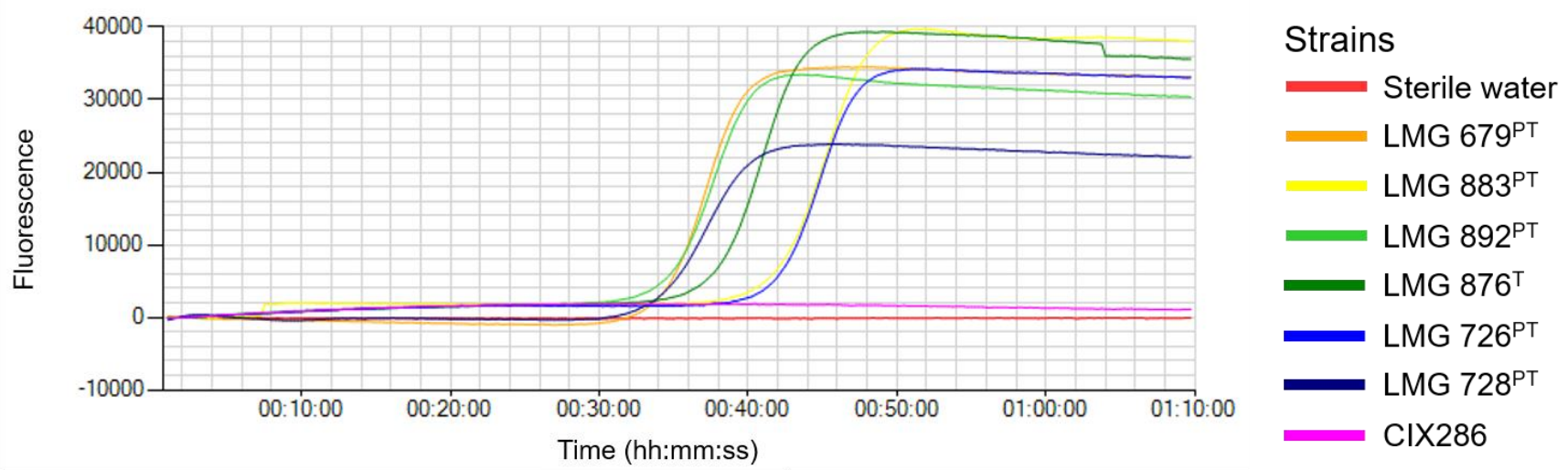
[a] Type (^{T}) and pathotype (^{PT}) strains.

[b] Sequence type (ST) based on the concatenated sequence of four loci (*rpoD, dnaK, fyuA*, and *gyrB*). Strains LMG 728^{PT} and CIX286 have not been assigned STs.

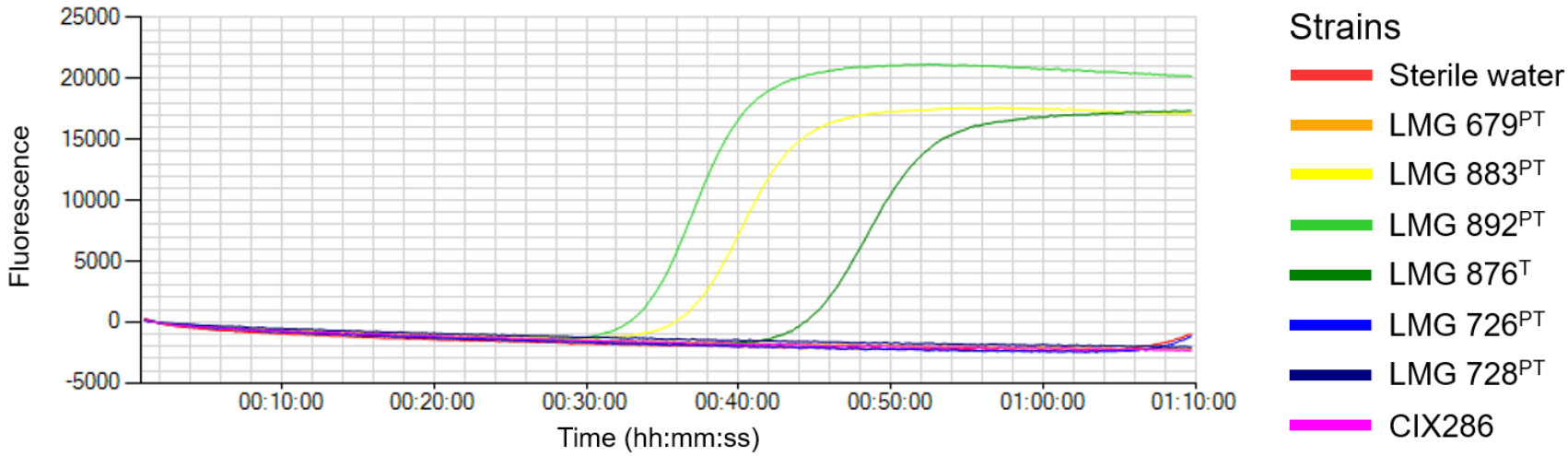
[c] BCCM/LMG = Belgian Co-ordinated Collections of Micro-organisms.



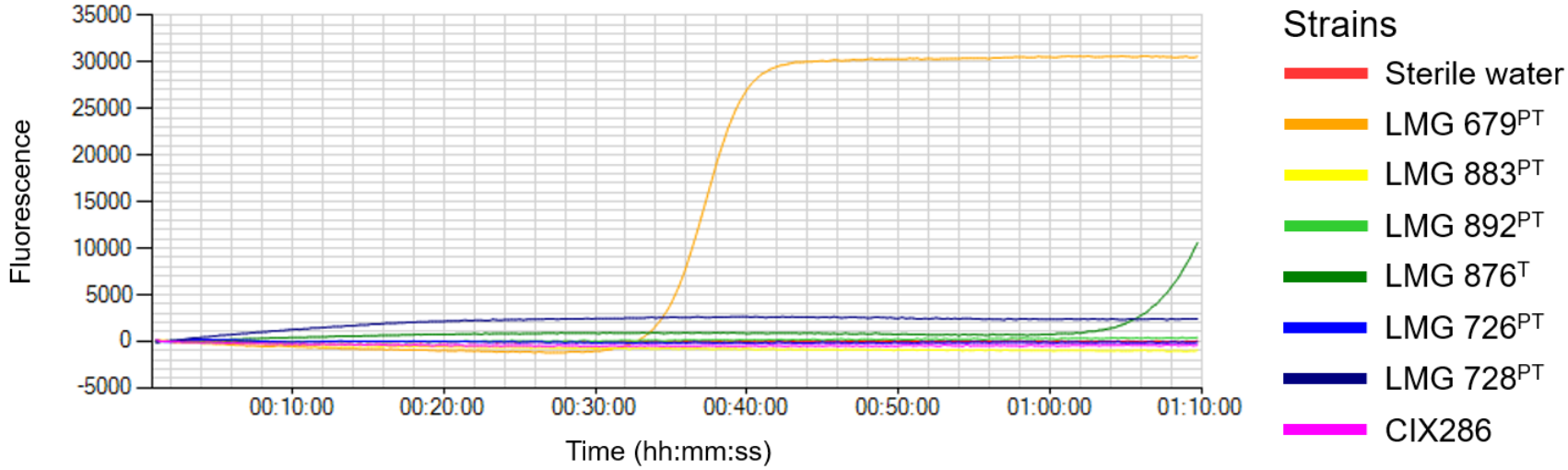
Appendix Figure 2.2. Character states at 4-5 days post inoculation depicted on barley and wheat seedling leaves when infiltrated with a mock saline solution, a known *X. translucens* pv. *translucens* (Xtt), and a known *X. translucens* pv. *undulosa* (Xtu) strain. Water-soaking (W) appeared as greasy, translucent lesions that expanded beyond the point of infiltration. Chlorosis (C) appeared as a yellow lesion that did not expand beyond the point of infiltration with no water-soaking symptoms. No response (NR) showed no host response.



Appendix Figure 2.3. Loop-mediated isothermal amplification (LAMP) results visualized as fluorescence across time on the Genie III instrument. This LAMP assay used the *gyrB*-Xt primer set designed to amplify all *X. translucens* pathovars (Langlois et al. 2017). Strains included in the assay were LMG 679^{PT} (*X. translucens* pv. *cerealis*), LMG 883^{PT} (*X. translucens* pv. *secalis*), LMG 892^{PT} (*X. translucens* pv. *undulosa*), LMG 876^{T} (*X. translucens* pv. *translucens*), LMG 726^{PT} (*X. translucens* pv. *graminis*), LMG 728^{PT} (*X. translucens* pv. *poae*), and CIX286 (*Xanthomonas* sp.).



Appendix Figure 2.4. Loop-mediated isothermal amplification (LAMP) results visualized as fluorescence across time on the Genie III instrument. This LAMP assay used the Xt-CLS primer set designed to amplify *X. translucens* pathovars *secalis*, *translucens*, and *undulosa* (Langlois et al. 2017). Strains included in the assay were LMG 679^{PT} (*X. translucens* pv. *cerealis*), LMG 883^{PT} (*X. translucens* pv. *secalis*), LMG 892^{PT} (*X. translucens* pv. *undulosa*), LMG 876^{T} (*X. translucens* pv. *translucens*), LMG 726^{PT} (*X. translucens* pv. *graminis*), LMG 728^{PT} (*X. translucens* pv. *poae*), and CIX286 (*Xanthomonas* sp.).



Appendix Figure 2.5. Loop-mediated isothermal amplification (LAMP) results visualized as fluorescence across time on the Genie III instrument. This LAMP assay used the Xt-Cerealis primer set designed to amplify *X. translucens* pv. *cerealis* (Langlois et al. 2017). Strains included in the assay were LMG 679^{PT} (*X. translucens* pv. *cerealis*), LMG 883^{PT} (*X. translucens* pv. *secalis*), LMG 892^{PT} (*X. translucens* pv. *undulosa*), LMG 876^{T} (*X. translucens* pv. *translucens*), LMG 726^{PT} (*X. translucens* pv. *graminis*), LMG 728^{PT} (*X. translucens* pv. *poae*), and CIX286 (*Xanthomonas* sp.).